ANNUAL REPORT 1997

AQUATIC WEED CONTROL RESEARCH

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
EXOTIC AND INVASIVE WEED RESEARCH UNIT

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INTRODUCTION

As of July this year, a major reorganization of ARS' western weed research programs has begun. The three units located in Reno, Davis and Albany have been combined under a single, new research unit, the Exotic and Invasive Weed Research Unit, led by Dr. Ray Carruthers. Ray is located at the Albany laboratory at the ARS Western Regional Research Center. This change exemplifies the growing importance ARS management places on developing solutions to the spreading problems caused by a variety of exotic weed- terrestrial, aquatic and riparian. The intent of the newly formed unit is to better coordinate and utilize scientific resources-both personnel and facilities- to build on existing strengths and develop new approaches for weed management.

Most or our cooperators recognize the need for a multifaceted strategies in developing new methods for aquatic weed management that minimize environmental impacts. The program at the Aquatic Weed Research Laboratory at Davis has had to sustain that goal with fewer and fewer resources over the past 6 to 8 years. At the same time, we have kept attuned to our state, federal and private sector clientele needs. Most recently, we have devoted considerable efforts in research and technology transfer to further the goals of the egeria control program lead by the Department of Boating a Waterways. While there are significant hurdles to overcome, particularly due to the highly variable flows of the Delta, concerns for threatened species and the constraints inherent in the highly divers uses of the Delta waters, I believe the restructuring of the western weed research unit can greatly enhance the potential for real progress. Although there is serious need to sustain and improve conventionally based aquatic weed management, it is clear that the long view must include renewed research on alternative strategies. These should not be seen as simply "non-chemical", but rather as a full spectrum of methods ranging form classical biological control to substrate-manipulation, competitive re-vegetation and natural-product based materials

This laboratory has, in fact, continually pushed into new areas including use of sterile grass carp, alterations of photoperiod-driven development, natural products (vinegar) and beneficial plant introductions and potential allelopathy (spikerushes). We are currently beginning to examine various microbial-based products that may (or may not) be effective alternatives to conventional algicides. The new organization might provide even more opportunities for these types of approaches. The current Annual Report is a summary of work conducted primarily during CY 1997, though some more recent results regarding the egeria project are also included. It contains diverse range of projects including both basic and applied research.

During the coming year, we'll strive to acquaint you with the new directions and combined resources, and research activities of the Exotic and Invasive Weed Research Unit. A good place to start is to visit its new website at:

http://wric.ucdavis.edu/exotic/exotic.htm

and our Aquatic Weed website at:

http://veghome.ucdavis.edu/AquaticWeed/About.htm

Lars W J Anderson

Lars W. J. Anderson, Ph.D,
Lead Scientist

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QUANTITATIVE COMPARISON OF VIABLE CELL COUNTS FOR SIX MICROBIAL WATER TREATMENT PRODUCTS

Graduate Student:	DuVall, Robert
Reporting Scientist:	Anderson, Lars

Objective: In recent years, microbial products and other non-pesticide water treatment products have become popular alternatives to algaecides and herbicides used in lake and pond management. The literature suggests that bacteria should be superior competitors for nutrients and in turn determine the abundance of algae. Microbial products are not regulated as algaecides and efficacy data that would otherwise be required by the California Department of Pesticide Regulation are not available. Reports on the successful use of microbial products are common. However, these reports are consistently anecdotal and subjective. Preliminary studies completed at this laboratory showed no significant decrease in algal growth and survival following the application of several microbial products when compared to non-treated controls. The purpose of this study was to confirm the viability of bacterial cells in several popular microbial water treatment products and to quantify and compare those cell counts (or colony forming units, CFU's).

Methods and Materials: Viable cell counts or colony forming units (CFUs) were obtained using the indirect method of serial dilution and agar plate spread. The average number of CFUs from six microbial products (Aqua-5™, a new formulation of LakePak™ WSP®, the old formulation of LakePak™ WSP®, Algae-Tron™, Biorestoration Formula-2™, and Biozyme™) were then compared. All six of the microbial products tested were in the form of a freeze-dried powder and are applied to lakes and ponds at a rate of approximately 5 lbs/acre-foot (2 mg/L). For each of the products, two milligrams of the powder was added to one liter of distilled water and stirred for five minutes before serially diluting and plating the suspension. The CFUs from water containing 2 mg/L of the microbial products were then compared to CFUs from untreated pond water and pond sediments.

Plate Count Agar (per liter: 9.0g agar, 5.0g pancreatic digest of casein, 2.5g yeast extract, and 1.0g glucose), Tryptic Soy Agar (per liter: 15.0g agar, 15.0g pancreatic digest of casein, 5.0g papaic digest of soybean meal, and 5.0g NaCl), Beef Extract Agar (15.0g agar, 5.0g peptone, and 3.0g beef extract) are three media commonly used for the culture of a wide range of microorganisms from the water. These media were used in the aforementioned quantification of viable cells and were also compared in order to determine the best medium for the culture of both microbial products and naturally occurring pond bacteria.

Results: Manufacturers of microbial products often guarantee that their products will contain a minimum bacterial count. These counts are usually expressed as a billion per gram or a trillion per pound. One of the products tested, LakePak™ WSP®, offers a guaranteed bacterial count of four billion CFUs per gram. This count is equivalent to 8,000 CFUs per ml in a 2 mg/L suspension. The average CFUs for the new formulation of LakePak™ WSP® was 3460 (Figure 1). However, the old formulation of LakePak™ WSP® consistently produced zero colonies at dilutions of 10^{-1} and 10^{-2} and could only be routinely detected in undiluted suspensions of 2 mg/L. Aqua-5 produced the highest average CFU count at 18,273.

The average CFU count from untreated pond water was 1600 per milliliter and 500,000 per milliliter from pond sediment (Figure 2). The Beef Extract Agar produced the largest CFU counts for the bacteria from the pond water and sediment while the Tryptic Soy Agar produced the largest CFU counts for the microbial products.

Figure 1

Viable cell counts or colony forming units (CFUs) from agar plates

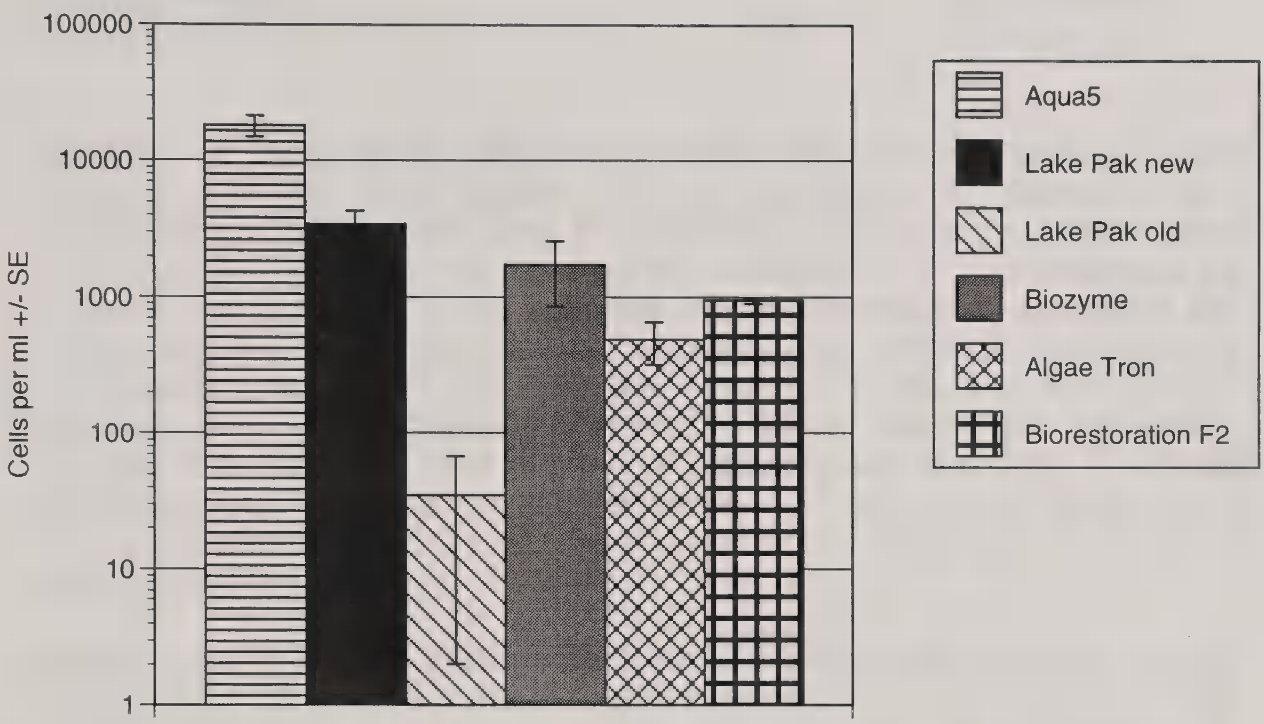
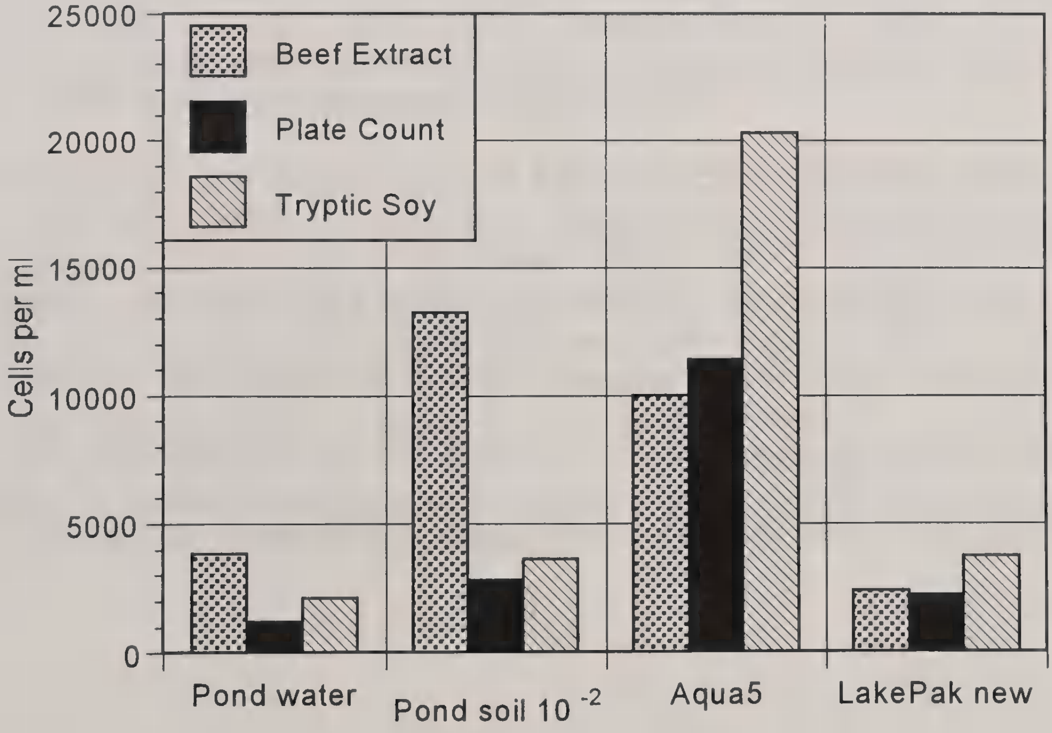


Figure 2

Cell counts on on three different media after inoculation with pond water, pond soil, Aqua-5 in distilled water (2mg/L) and new Lake Pak in distilled water (2mg/L).



EFFECTS OF FIVE MICROBIAL WATER TREATMENT PRODUCTS ON PHYTOPLANKTON

Graduate Student: DuVall, Robert
Reporting Scientist: Anderson, Lars

Objective: In recent years, microbial products and other non-pesticide water treatment products have become popular alternatives to algaecides and herbicides used in lake and pond management. The literature suggests that bacteria should be superior competitors for nutrients and in turn determine the abundance of algae. Microbial products are not regulated as algaecides and efficacy data that would otherwise be required by the California Department of Pesticides Regulation are not available. Reports on the successful use of microbial products are common. However, these reports are consistently anecdotal and subjective. The purpose of this study was to test the hypothesis that concentrated blends of freeze dried bacteria and enzymes can be applied to lakes and ponds in order to clear green water, inhibit algae growth or improve water clarity or quality.

Methods and Materials: Water from a ten acre urban lake in Davis, CA was collected from 10 cm below the surface and transferred to 35 separate 60 liter barrels located outdoors at the Aquatic Weed Control Research Laboratory. The water in this lake contained a bloom population of the green alga, *Chlorella sp.* Chlorophyll *a* concentrations in this lake were measured several times by this laboratory in 1997 and were consistently in the range of 100 to 250 mg/m^3.

Chlorophyll *a* concentrations were determined from 25 ml samples collected in the top 10 cm of water. These samples were filtered on glass fiber filters and the filters were then placed in 10 ml DMSO to extract the Chlorophyll. The optical densities of extracts were then measured on a Beckman DU-64 spectrophotometer and used to calculate chlorophyll *a* concentrations in the water using the trichromatic method for chlorophyll.

Five replicates were used for each of the five microbial products tested (Aqua-5™, LakePak™ WSP®, Algae-Tron™, Biorestoration Formula-2™, and Clear Pond™), the positive control (Hydrothol 191™) and the negative control (no treatment). The water in the barrels was allowed to acclimate overnight and pre-treatment chlorophyll *a* concentrations were determined. The five microbial products were then applied at the recommended rate of 3 lbs./acre-foot and the Hydrothol was applied at 1 ppm. Post-treatment chlorophyll *a* concentrations were then determined at 5 hours, 24 hours, 3 days, 5 days, 10 days, 15 days and 30 days. A maintenance dosage of one pound per acre foot every two weeks is recommended for most of the microbial products tested and was applied for all of

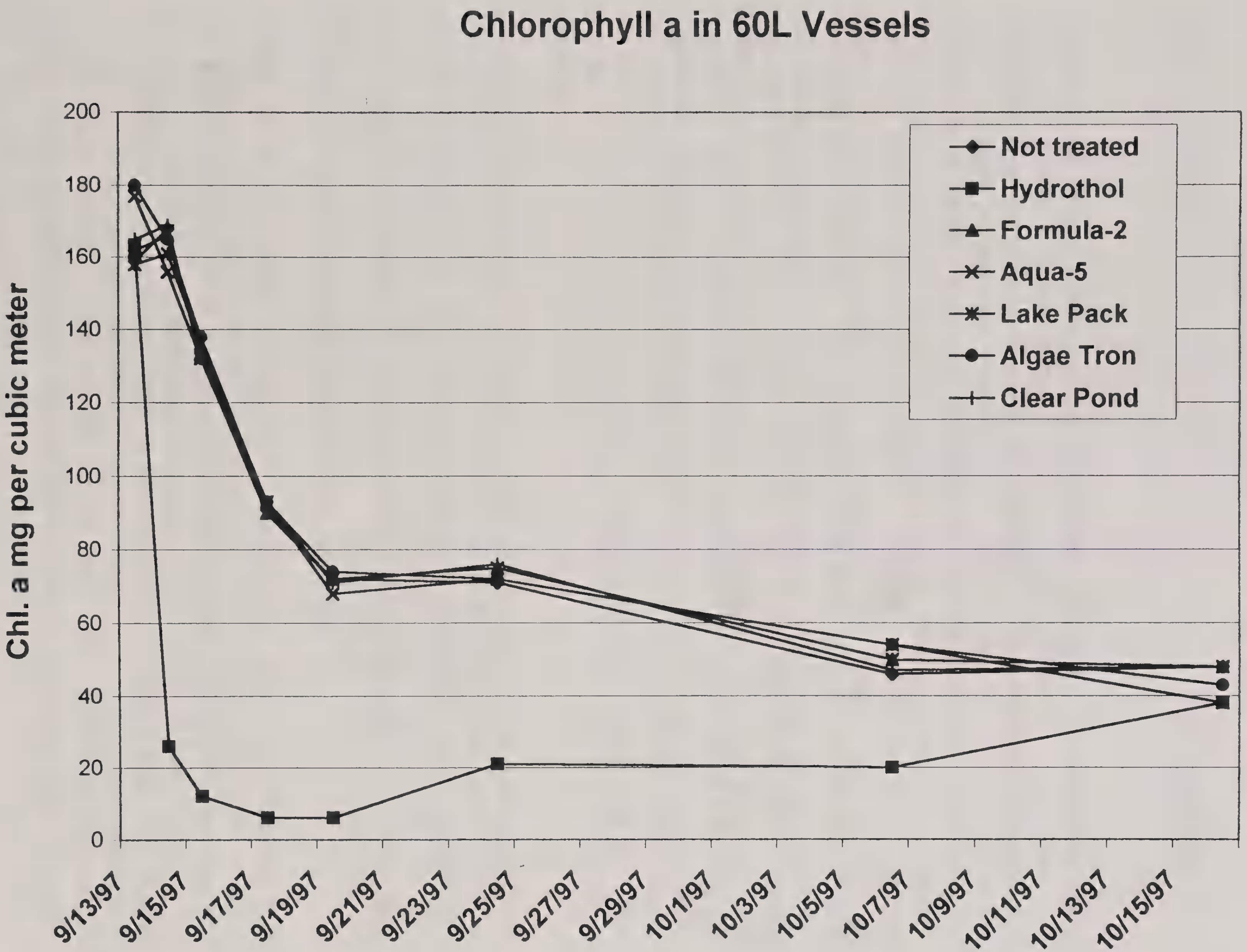
them on the 14th day of the study. A maintenance dosage of the Hydrothol was not applied.

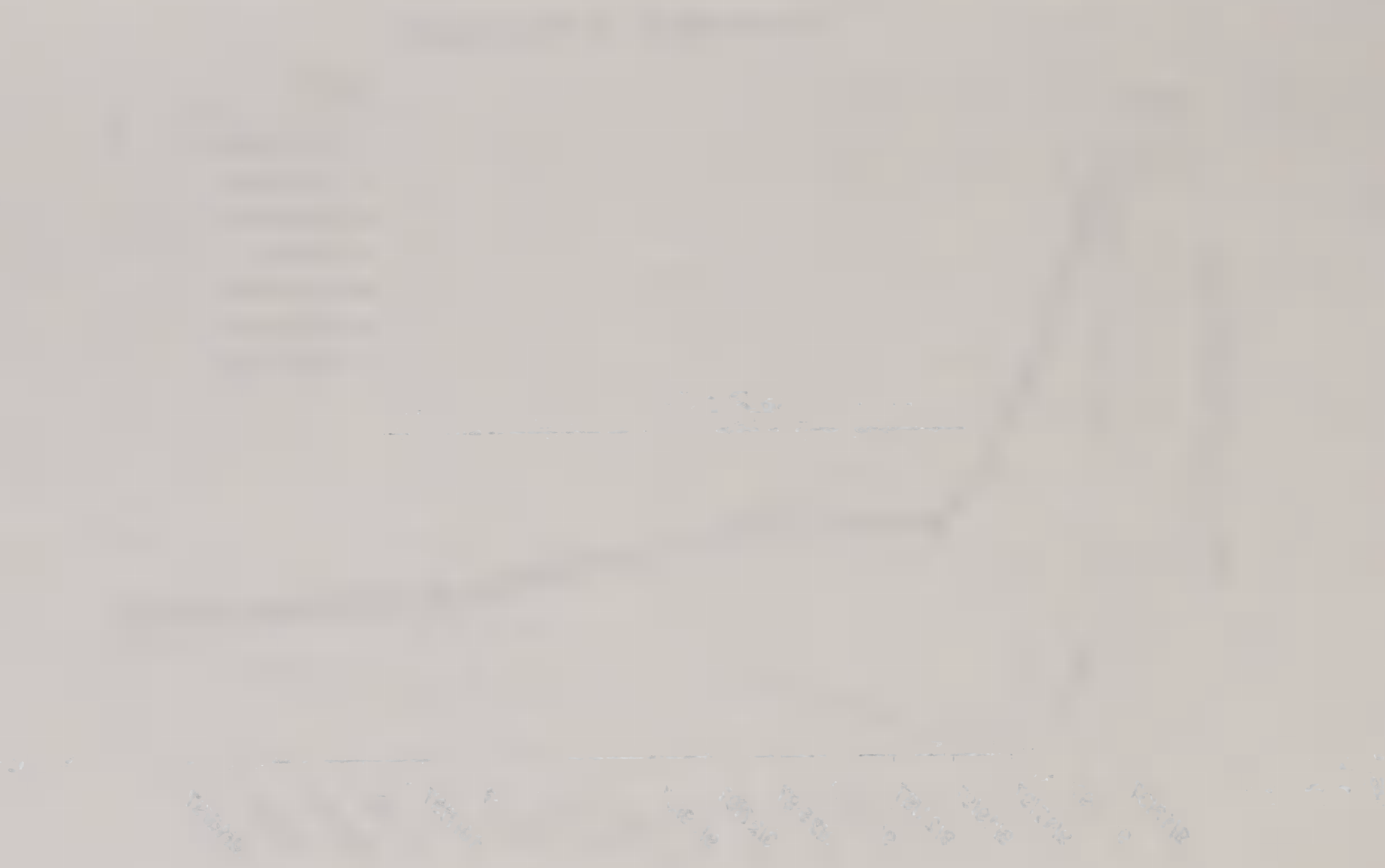
On the ninth day of the study 12 liters of well water were added to each barrel to make up for evaporation loss. On the 14th day six liters were again added. Conditions in the barrels were similar to those of the lake. Dissolved oxygen in the lake, the barrels treated with the microbial products and the non-treated control barrels remained similar, near 7 mg/L. However, DO in the barrels treated with the Hydrothol was only 50% that of all the other barrels. The temperature fluctuated in the barrels from a maximum of 32° C to a minimum of 14° C while the temperature of the lake fluctuated less and remained around 22° C. The pH of the lake and all the barrels remained high, from 9.0 to 9.4 throughout the study.

Results: Chlorophyll *a* concentrations in the lake declined from 170 mg/m^3 on September 13th to 110 mg/m^3 on September 24th. A similar but slightly more rapid decline was observed in the laboratory barrels, except for the five barrels treated with Hydrothol 191™. In the Hydrothol treated barrels there was an immediate reduction of chlorophyll, and 24 hours after treatment chlorophyll levels were essentially zero. The reduction of DO observed in the Hydrothol barrels corresponds with the measured loss of photosynthetic activity. The results of the Hydrothol 191™ treatments are similar to those obtained in separate studies conducted by this laboratory.

All five of the microbial products tested produced results equivalent to the non-treated controls (Figure 1). The results of this study indicate that under the conditions of this lake system, concentrated bacteria and enzyme blends do not clear green water or inhibit algae growth. Follow-up studies are needed to determine if the high pH of this eutrophic lake reduced the effectiveness of the microbial products tested. Additional studies are also needed to determine if bacterial populations in a lake can be increased by the addition of more bacteria. Bacterial populations may be limited by resources or the chemical, physical and biological characteristics of the lake environment and not limited by a lack of bacterial propagules.

Figure 1


Chlorophyll a in 60L Vessels
Chl. a mg per cubic meter
200
180
160
140
120
100
80
60
40
20
0
9/13/97
9/15/97
9/17/97
9/19/97
9/21/97
9/23/97
9/25/97
9/27/97
9/29/97
10/1/97
10/3/97
10/5/97
10/7/97
10/9/97
10/11/97
10/13/97
10/15/97
Not treated
Hydrothol
Formula-2
Aqua-5
Lake Pack
Algae Tron
Clear Pond



EFFECT OF A BIOLOGICAL LAKE CLARIFIER ON ALGAE GROWTH MEASURED AS THE CONCENTRATION OF CHLOROPHYLL *a*

Graduate Student:	DuVall, Robert
Reporting Scientist:	Anderson, Lars

Objective: In recent years, microbial products and other non-pesticide water treatment products have become popular alternatives to algaecides and herbicides used in lake and pond management. The literature suggests that bacteria should be superior competitors for nutrients and in turn determine the abundance of algae. Microbial products are not regulated as algaecides and efficacy data that would otherwise be required by the California Department of Pesticide Regulation are not available. Reports on the successful use of microbial products are common. However, these reports are consistently anecdotal and subjective. The purpose of this study was to test the hypothesis that concentrated blends of freeze dried bacteria and enzymes can be applied to lakes and ponds in order to clear green water, inhibit algae growth or improve water clarity or quality. Preliminary studies completed in October 1997 indicated that there was no significant difference in algal growth and survival following the application of several microbial products when compared to non-treated controls. One potential explanation for these results was that the pH of the water used in the study was too high (9.0 or higher). The purpose of this study was to further test the efficacy of microbial products in controlling algae under conditions of lower pH.

Methods and Materials: Water for this study was prepared by lowering the pH of domestic tap water to 7.0 with 15 ml of 5N HCL in 70 liters of tap water and then removing chlorine from the water with sodium thiosulfate. Small plastic pots were used to combine 670 ml of the modified tap water and 140 ml of water collected from an outdoor aquatic plant vault which contained a bloom of mixed algae dominated by *chlamydomonas sp.* A final pH of 7.25 resulted from the addition of the algal inoculum to the modified tap water.

A total of 60 pots were placed in the greenhouse. One third of these pots received an additional 40 ml of Hoagland's nutrient solution (5% total volume), one third received 4 ml of Hoagland's nutrient solution (1/2%) and the final one third did not receive any additional nutrients (0%). Each of the three nutrient concentrations then received either a 100x labeled rate (150 mg), 10x labeled rate (15 mg), normal labeled rate (2 mg) or a zero rate (non-treated) application of LakePak™ WSP®. The normal labeled rate was considered the highest manufacturers recommended initial application rate of 5 lbs./acre-foot (1.8 mg/L). The 60 pots (five replicates and 12 treatments) were then arranged in a completely randomized design in the greenhouse.

Chlorophyll *a* concentrations were determined at three and 15 days after treatment and dry biomass was measured 120 days after treatment. At three days, 50 ml water samples were taken from the middle of the pots. After fifteen days, mixing was required to homogenize the algae in the pots since thick mats of filamentous algae had developed on the surface of some pots. Samples were then collected by removing 50 ml of water from the middle of the pots. Water samples were filtered on glass fiber filters and the filters were then placed in 10 ml DMSO to extract the Chlorophyll. Optical densities of the extracts were then measured on a Beckman DU-64 spectrophotometer and used to calculate chlorophyll *a* concentrations in the water using the trichromatic method for chlorophyll.

Results: After three days, chlorophyll *a* concentrations in the pots significantly increased ($P < .05$) for both the treatments receiving added nutrients and high rates of the microbial product (Figure 1). After 15 days only the treatments receiving the highest addition of nutrients maintained a significantly higher concentration of chlorophyll *a*, and within the 5% treatments, the pots receiving the 100x microbial products were again significantly higher ($P < .01$) in chlorophyll *a* concentrations compared to the non-treated controls. Biomass dry weight also increased with the addition nutrients and microbial products (Figure 3).

The results of this study support earlier results that showed the addition of concentrated bacteria and enzyme blends do not clear green water or inhibit algae growth. It was observed that filamentous algae was nearly absent from the pots treated with the 100x microbial product rate while it formed dense mats at the tops of all the other pots. Additional studies are needed to determine if the rapid growth of the phytoplankton inhibited the development of filamentous algae or if some other explanation exists. Another observation in the 100x pots was the attraction of metallic fragments on the magnetic stir bar used to mix water in the pots. Further investigation revealed a high number of these fragments in the dry product. The addition of iron or other nutrients such as nitrogen or phosphorus in the microbial product tested may explain the observed stimulation of algal growth.

Figure 1

Chlorophyll a Concentration in One Liter Greenhouse Vessels Three Days After Treatment with Lake Pack

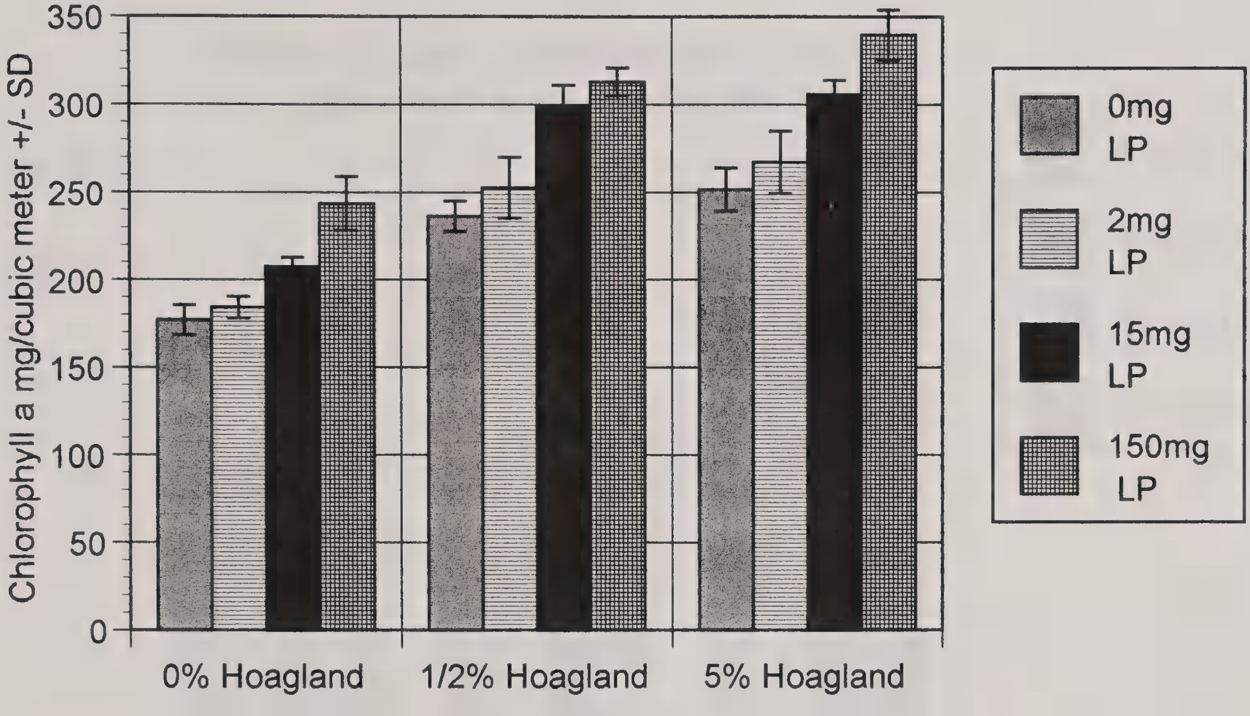


Figure 2

Chlorophyll *a* Concentration in One Liter Greenhouse Vessels Fifteen Days After Treatment with Lake Pack

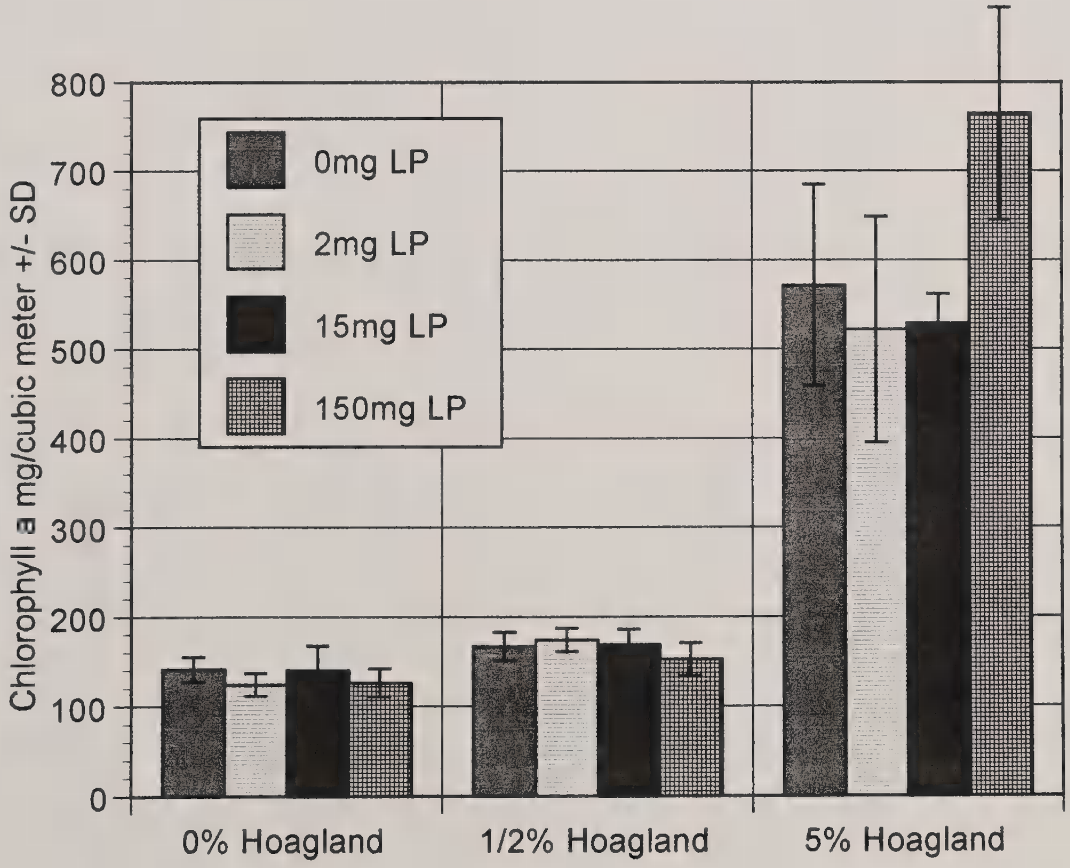
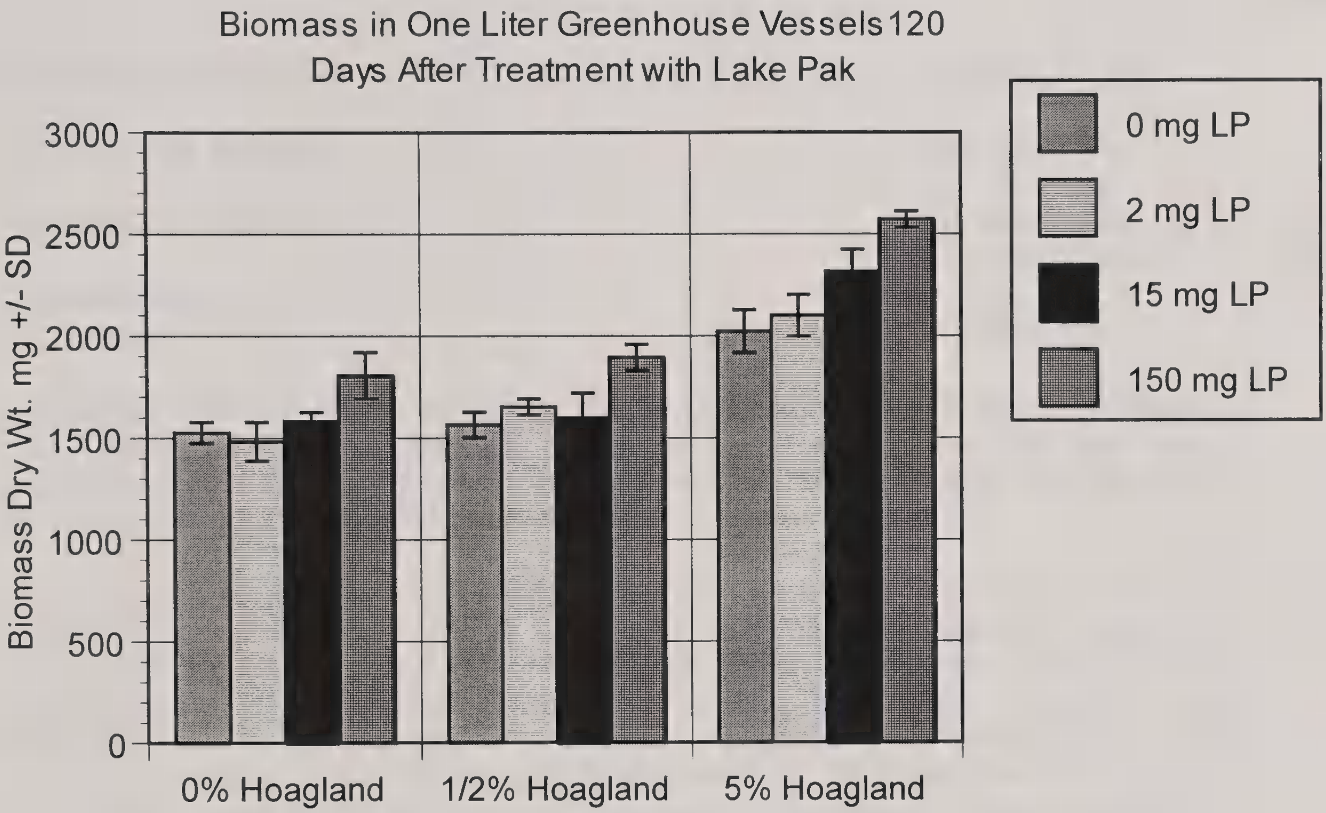


Figure 3


Biomass in One Liter Greenhouse Vessels 120 Days After Treatment with Lake Pak
Biomass Dry Wt. mg +/- SD
0
500
1000
1500
2000
2500
3000
0% Hoagland
1/2% Hoagland
5% Hoagland
0 mg LP
2 mg LP
15 mg LP
150 mg LP

EFFECTS OF MECHANICAL HARVESTING ON FRAGMENT PRODUCTION AND VIABILITY IN *Egeria densa*

Reporting Scientist:	Anderson, Lars
Postdoctoral Scientist:	Gee, Doreen
Associated Technicians:	Pirosko, Chris Holmberg, Debe
Graduate Student:	Duvall, Rob
High school Intern:	Morris,Noah

Objective: As part of the California Dept. of Boating and Waterways egeria control program, data on impacts of various management methods are being assessed. This study was conducted to determine numbers, size and viability of shoot-fragments that result from typical egeria harvest operations.

Methods and Materials: Plots of ca. 1 acre were harvested in the fall of 1997 by Dept. of Water Resources (DWR) personnel at three sites in the Sacramento/ San Joaquin Delta: Sand Mound Slough (9/21/97), Seven Mile Slough (9/23/97) and White Slough (9/28/97). Floating fragments of egeria were collected from boats pre- and postharvest with small fish nets (18" by 15" opening with 0.25" mesh) for specific time intervals. All fragments within range of the boats were taken as the boat traversed "downstream" (based upon tidal flows) from each plot. Collected fragments were brought to the Aquatic Weed Control Facility where the numbers and lengths were recorded. To assess viability, fragments in three size classes (9, 18, 23 cm) containing intact apical meristems were placed in 1 gal. glass jars with 2.5 l Delta water taken from the harvest site. Two configurations of fragments were used: (1) three, same-size class fragments were in one container (= one replicate); and (2) one of each size class was placed in one container (= one replicate). Five replicates were used for each configuration. All containers were maintained in a growth chamber at 17C, LD: 14/10 under ca. 165 µm /m/sec cool-white light. At the beginning and 21 to 24 days after introduction to the growth chamber, numbers of lateral shoots, roots, lengths of laterals shoots, and fresh weights were recorded.

Results: Numbers of fragments collected during the harvests varied considerably and ranged from several hundred to over 2,000 (Figure 1a). The "background" of floating fragments was generally less than 100. Most fragments were from 1 to 20 cm long (e.g. Figure 1b), although some exceeded a meter. It is clear that a rapid increase in presence of fragments is associated with harvesting in all three sites.

There were small increases in the fresh weight of fragments of all three size classes between harvest and 21 days after planting (DAP) (Figure 1c). However, there was a sharp increase in both new lateral shoot production, length of laterals and new root production in all size classes (Figs. 2-5). These

data indicate that nearly all fragments would be capable of initiating new populations of egeria if they became lodged in sediment with adequate nutrients and stability. There appeared to be an inhibitory effect on both new lateral shoot initiation and root initiation when all three size classes were maintained in a one container (Fig. 3, 5). This interesting response may be due to decreased light, lowered nutrients, or other indirect pgr effects related to the length of the apical fragments. However, if it were light or nutrient limitations, one would have expected reduced shoot initiation on the larger (23cm) size fragments even when they were cultured alone.

Figure 1a.

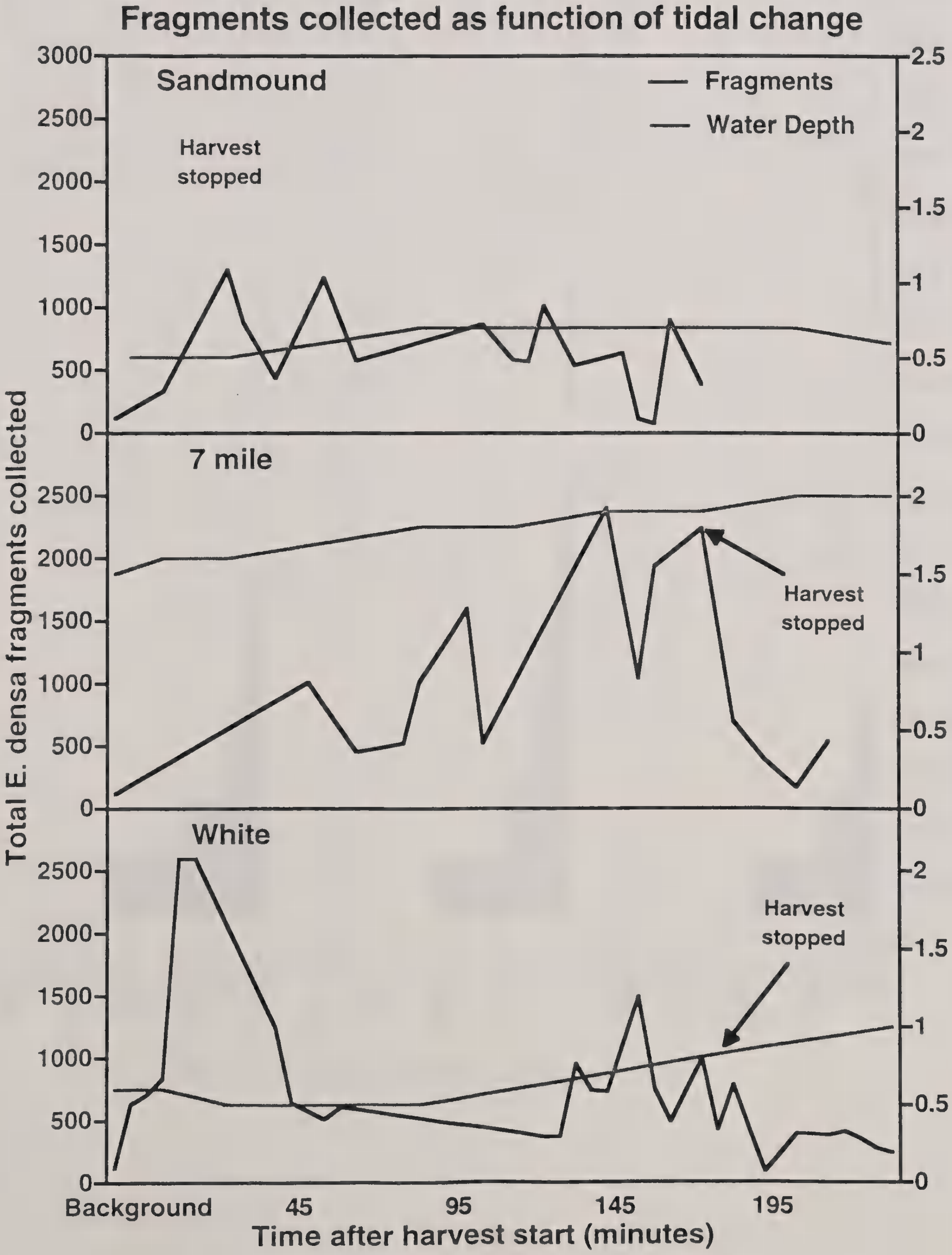


Figure 1b. *Egeria densa* fragments collected after mechanical harvesting in Sandmound Slough on September 21, 1997

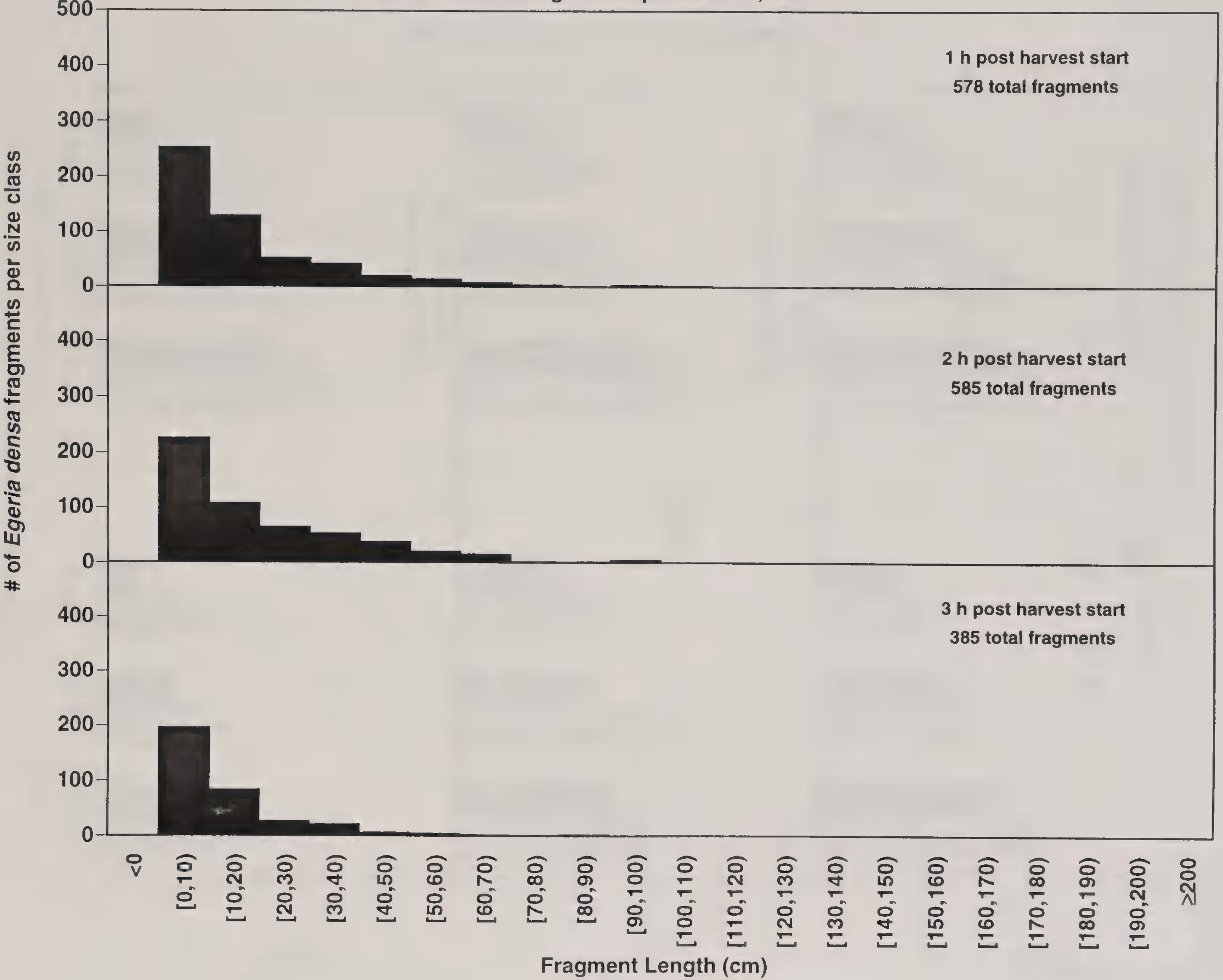


Figure 1c. *Egeria densa* fragment re-growth in delta water in growth chamber after mechanical harvest

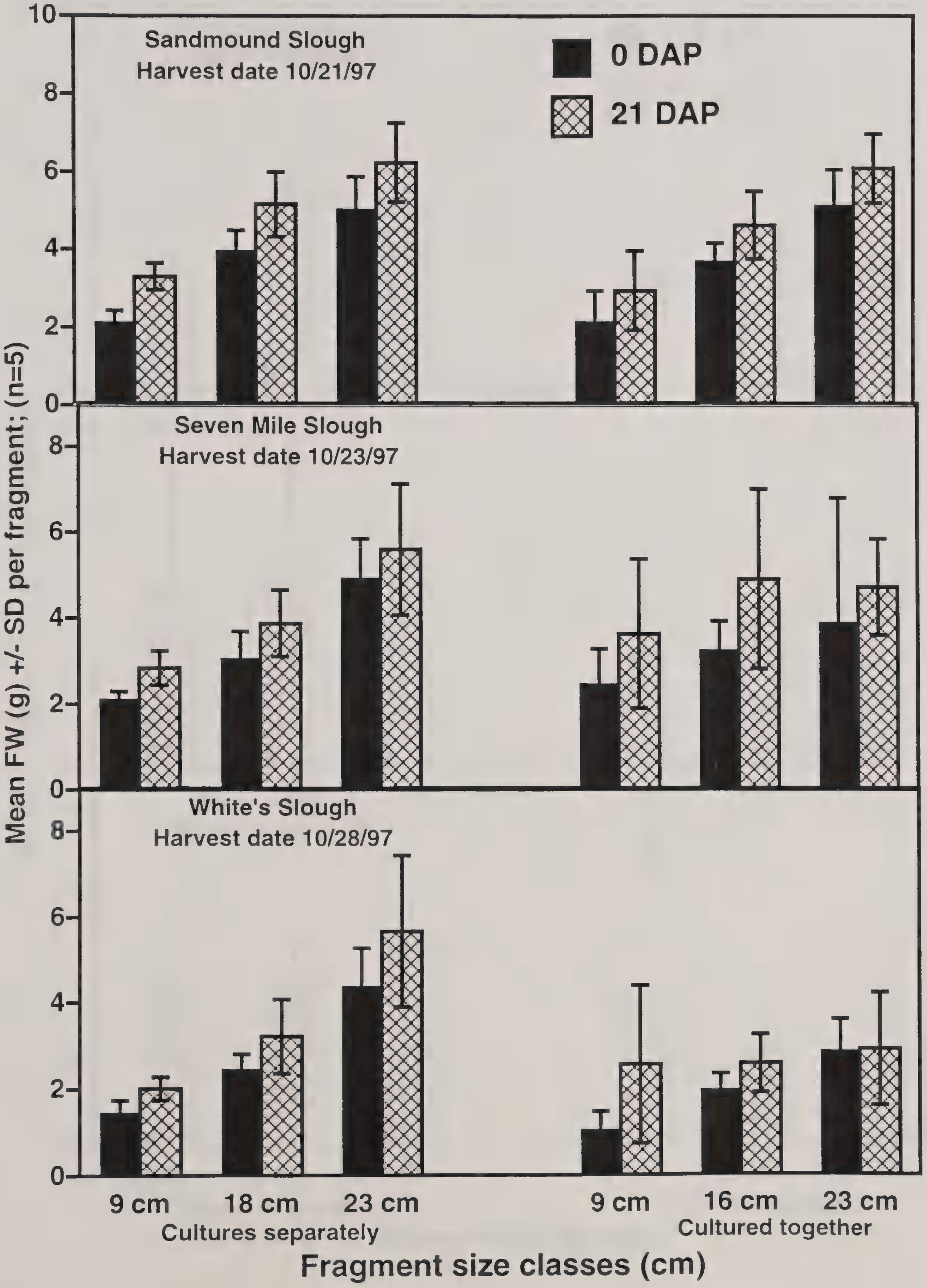


Figure 2. *Egeria densa* lateral shoot recovery in growth chamber after mechanical harvest

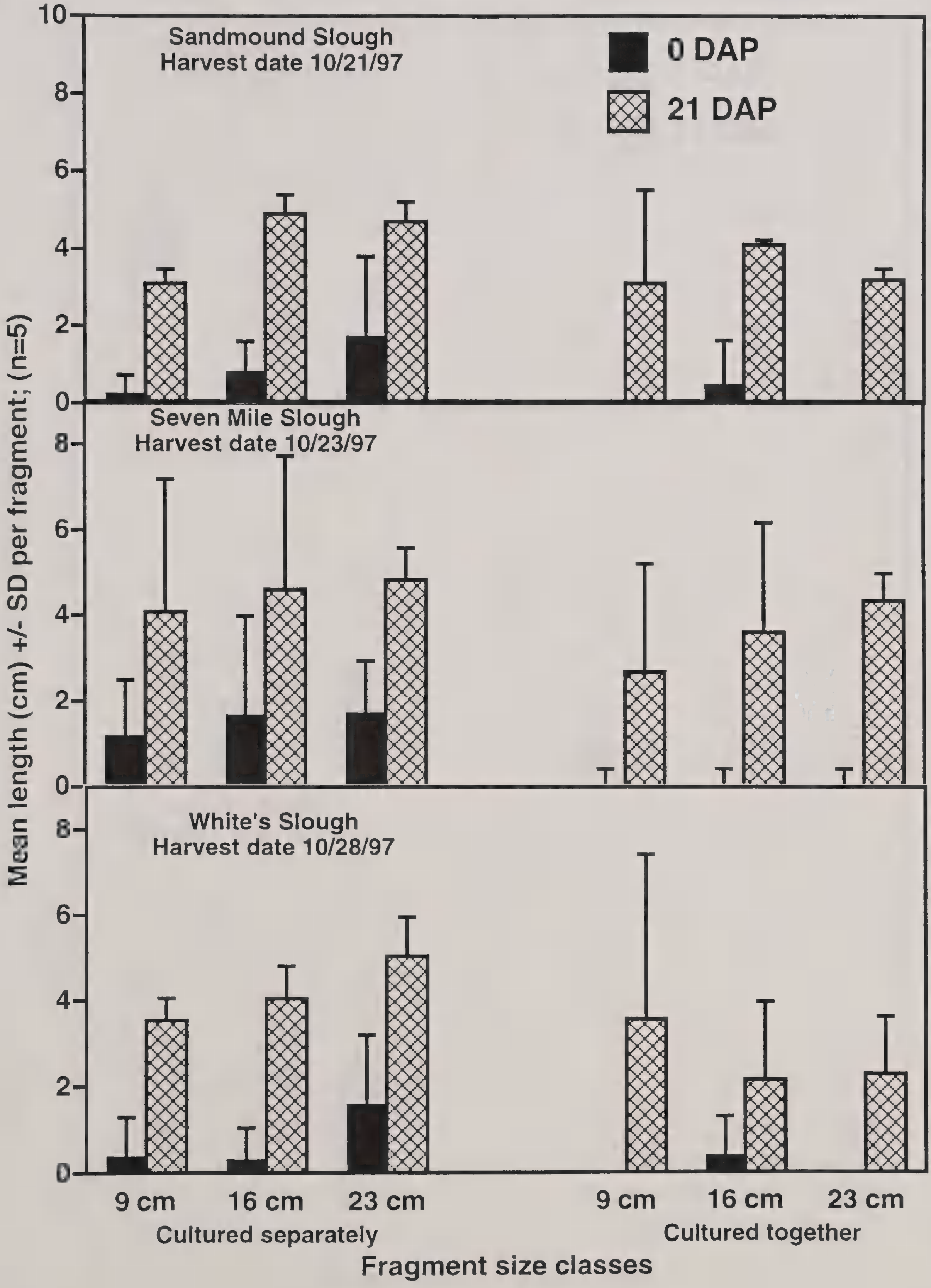


Figure 3. *E. densa* lateral shoot production per original fragment in growth chamber after mechanical harvest

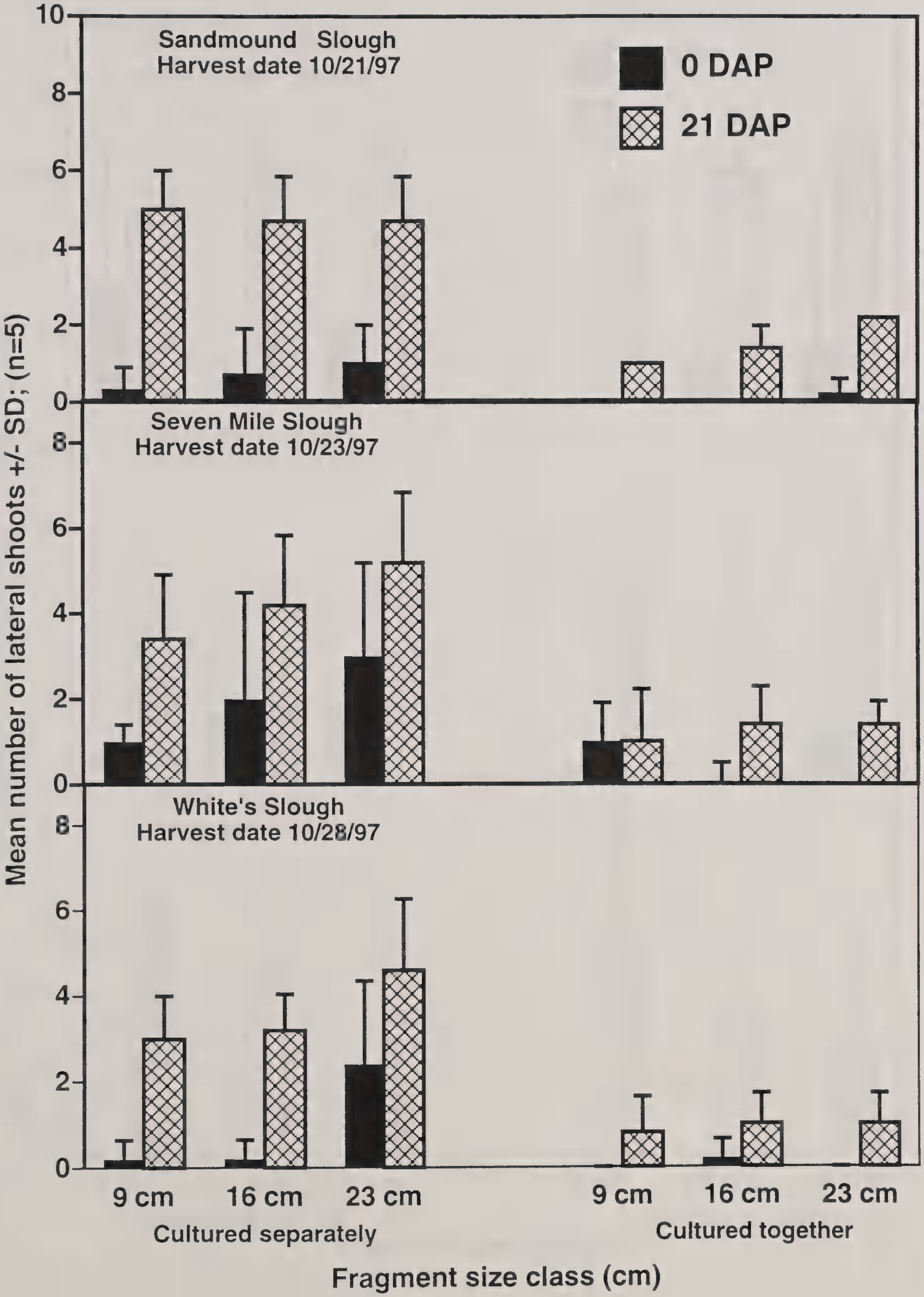


Figure 4. *E. densa* adventitious root recovery in growth chamber after mechanical harvest

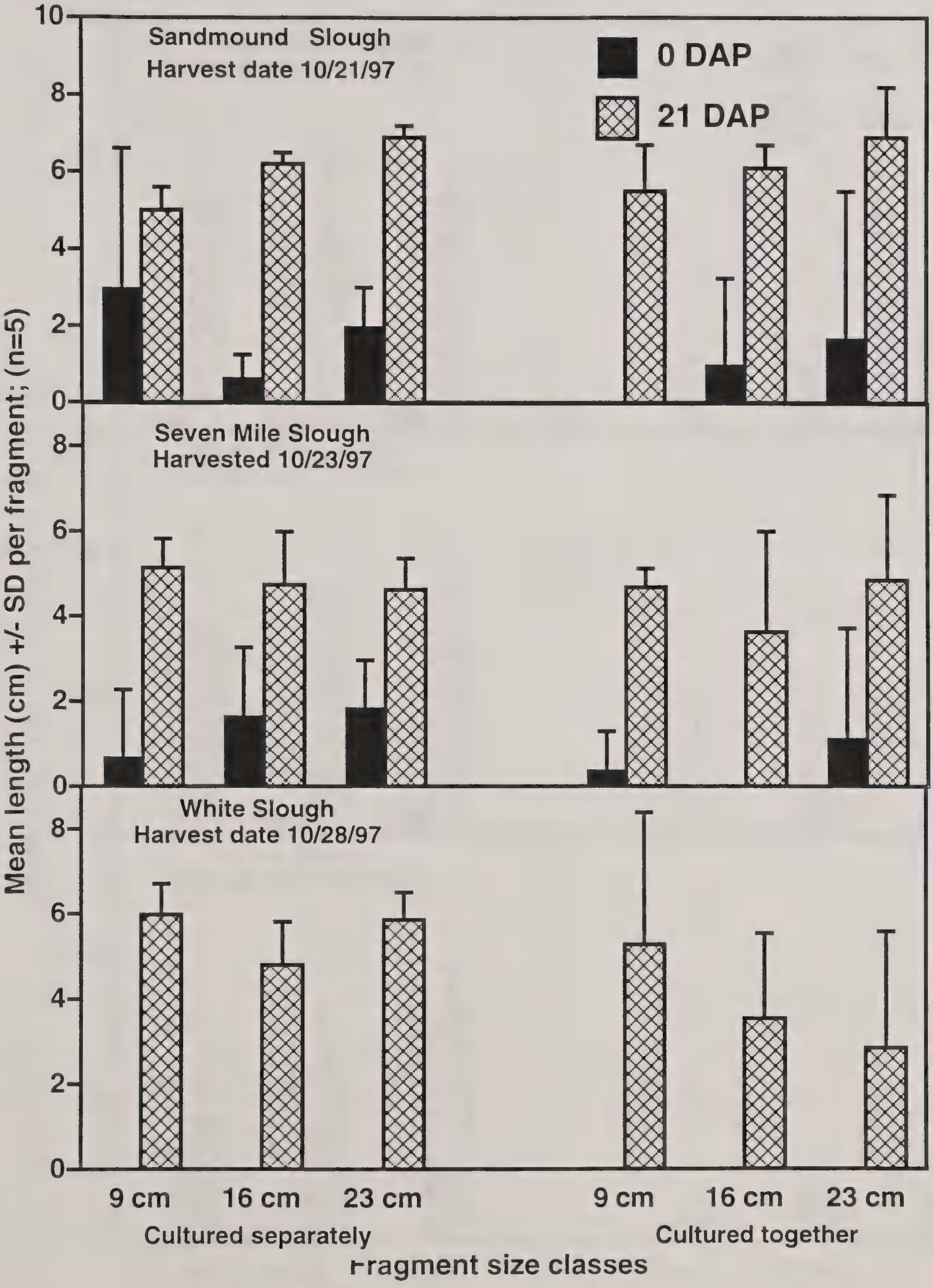
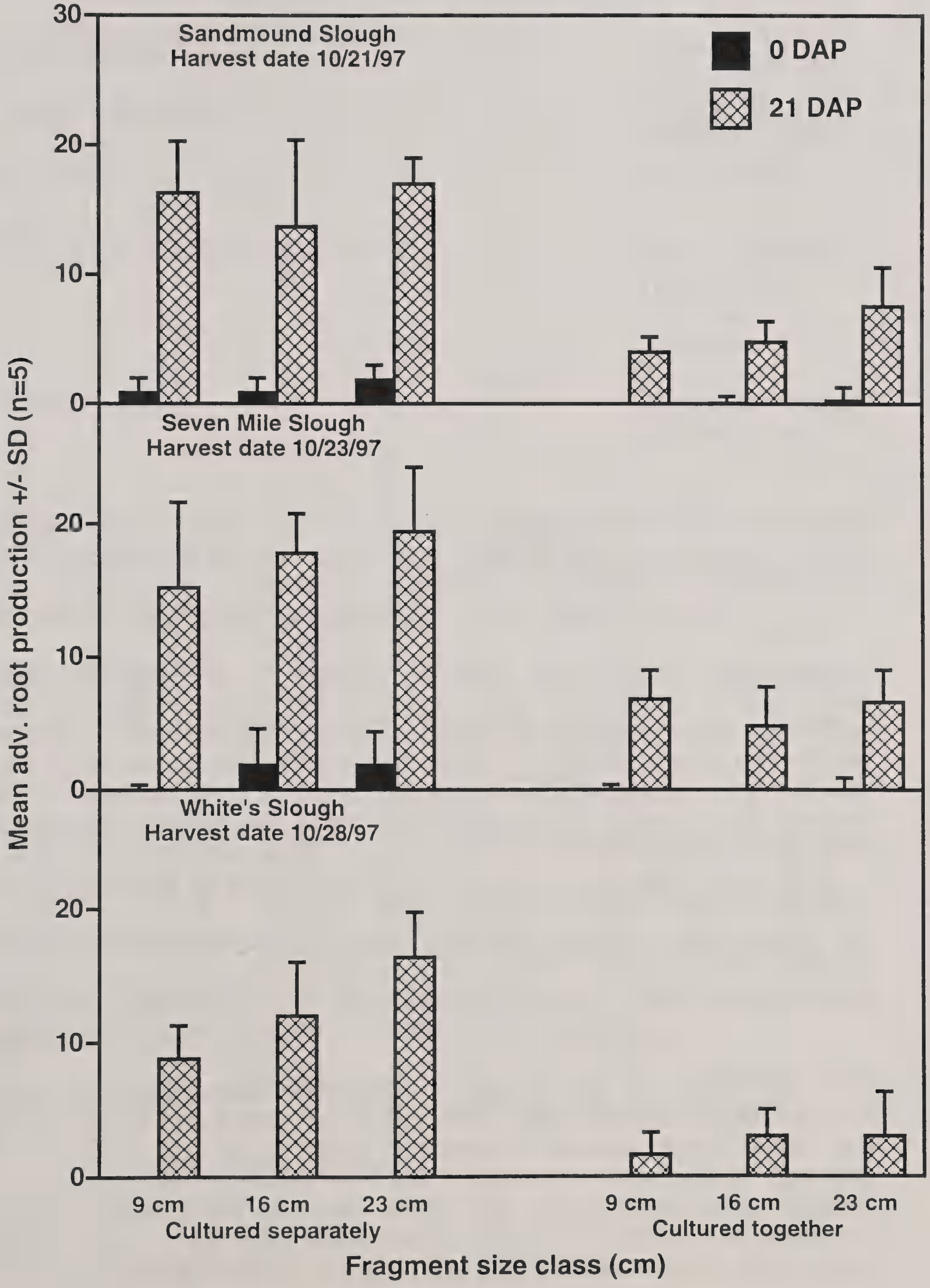


Figure 5. *E. densa* adventitious root production per original fragment in growth chamber after mechanical harvest



DISSIPATION OF FLURIDONE FOLLOWING APPLICATIONS OF SONAR TO THREE EGERIA-INFESTED SITES IN THE SACRAMENTO-SAN JOAQUIN DELTA

Reporting Scientist:	Anderson, Lars
Associated Technicians:	Pirosko, Chris Holmberg, Debe
Student Intern:	Morris, Noah
Cooperators: California Dept. of Boating and Waterways)	Vanway, Valerie Thalken, Pat Arts, Tim Waltz, Don
SePro Corporation:	Cockreham, Steve Littlefield, Terry

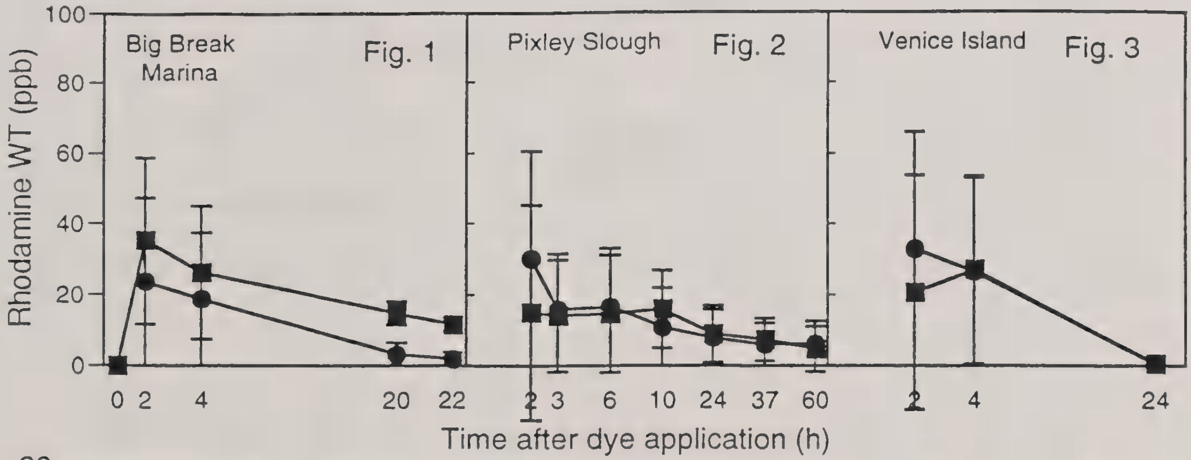
Objective: High velocity water flow and tidal exchanges in the Delta make the use of herbicides difficult, particularly those requiring long contact time such as Sonar. This study was undertaken to determine if sufficient concentrations of fluridone from Sonar persist for potential control of *Egeria densa*.

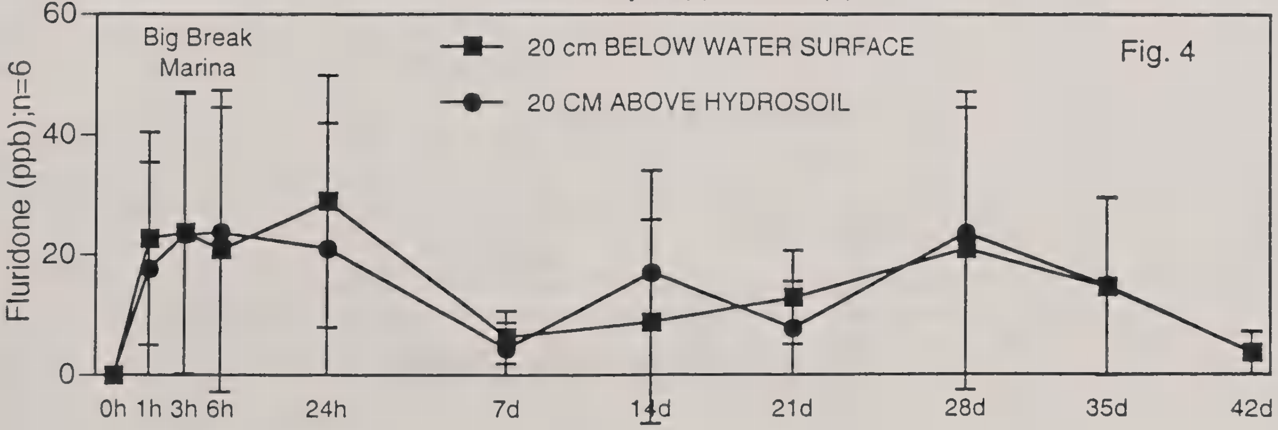
Methods and Materials: To estimate residence time of water and potential dilution of applied Sonar, initial studies using Rhodomine WT dye were conducted at Big Break Marina, Pixley Slough and Venice Island and White Slough. Dye was applied with weighted hoses from an air boat to produce an initial concentration of ca. 30 ppbw. Movement and dissipation of the dye was monitored with a Turner field flurorometer equipped with a flow-through cuvette. Subsequently, Sonar was applied by air boat twice per week either as the SRP formulation (Venice Isl. and Frank's Tract) or liquid 4 AS (Big Break Marina). Target levels were 10 to 20 ppbw. Water sampling stations were established inside and outside the treated plot area. Water was sampled at two depths: 20 cm below the surface and 20 cm from the bottom before and at several posttreatment intervals after applications had begun. Water samples were shipped to SePRO and analyzed via FasTest immunoassays.

Results: The results of dye monitoring are shown in Figs. 1-3 and indicate that Big Break Marina had a low rate of dissipation, while Venice Isl. had the most rapid loss of dye (e.g. within 24h of application). Although Pixley Slough had the most favorable residence time (up to 60h), the proximity to non-stop irrigation precluded this site from being used during the growing season. Fluridone levels at the three sites used are shown in Figures 4-6. From the data, it would appear that both Frank's Track and Big Break Marina might have

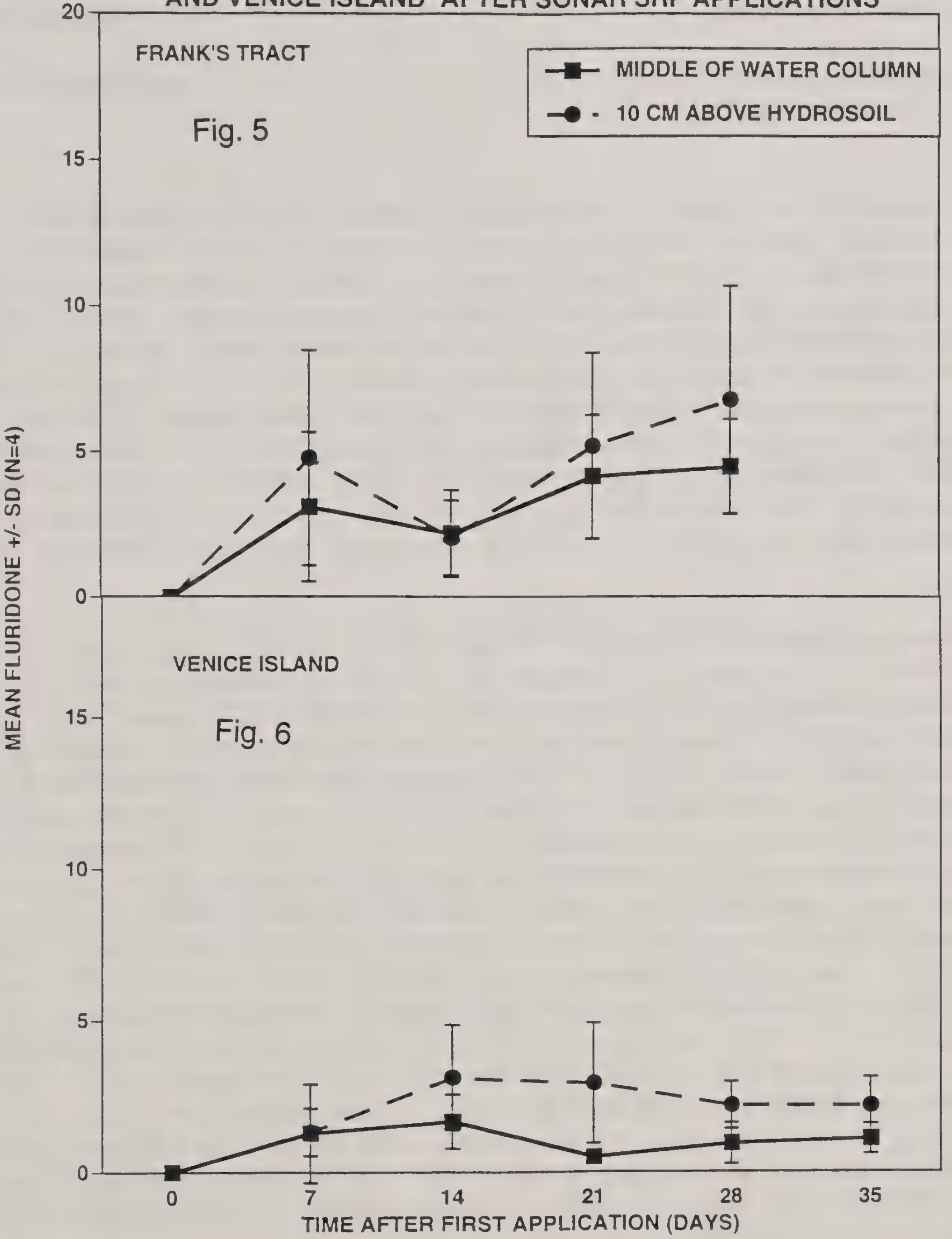
sufficiently sustained levels of fluridone to affect some control of egeria. However, the high flows at Venice Isl. caused a rapid flushing of the herbicde, at least at points in the water column ca. 20 cm above the sediment. NOTE: Some chlorosis was observed at Frank's Track and Big Break Marina. Final Biomass data has not been obtained for all sites.

These data suggest that at least for some areas not directly in the main river-flow (as is Venice Isl.), it may be possible to achieve concentrations of fluridone for long enough duration to affect growth of egeria. Additional studies are planned for winter 1998 and spring 1999 to build upon these preliminary findings.





FLURIDONE DISSIPATION IN TREATMENT AREA OF FRANK'S TRACT AND VENICE ISLAND AFTER SONAR SRP APPLICATIONS



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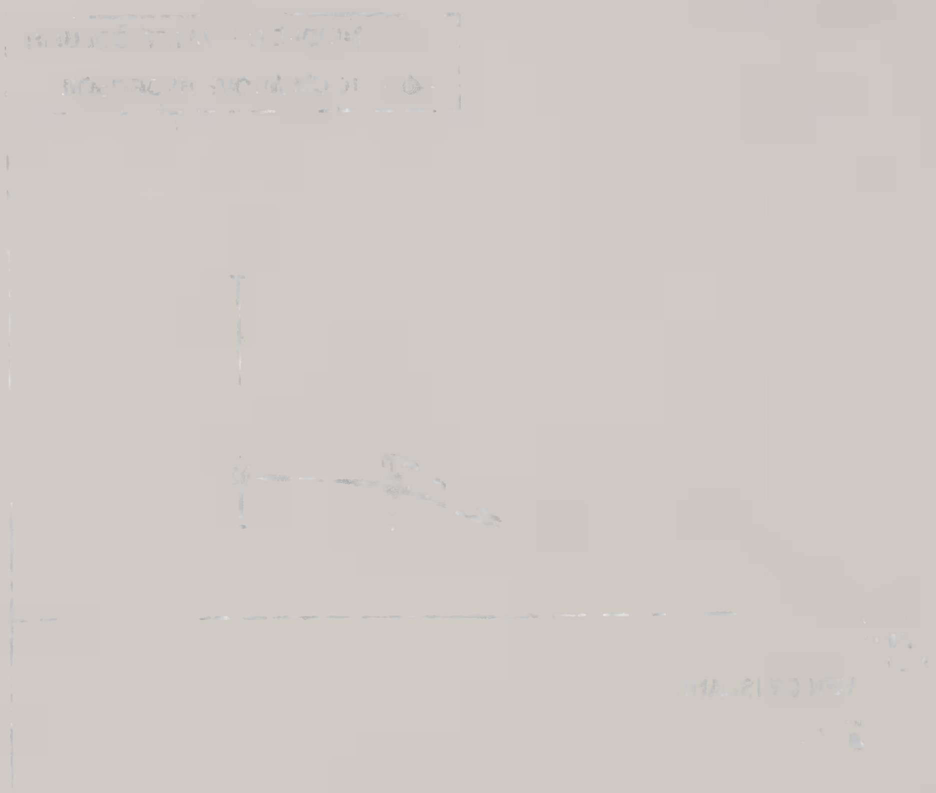


RE-GROWTH OF EURASIAN WATERMILFOIL *(Myriophyllum spicatum)* FOLLOWING SIMULATED HARVESTING AND EXPOSURE TO SONAR

Reporting Scientist:	Anderson, Lars
Associated Technicians:	Fellows, Suzanne Pirosko, Chris

Objective: The herbicide Sonar requires long-duration exposure (4 to 6 weeks) at low concentrations (10 to 25 ppbw). Optimal efficacy is usually achieved when target plants are growing rapidly (e.g. early spring). Rapid growth may be induced by cutting upper-canopy biomass, for example by mechanical harvesting. This tends to stimulate new lateral shoot formation and provide new growing point where Sonar can be taken up and block carotenoid synthesis. In addition, harvesting alone generates large numbers of plant fragments in many target species which can spread weed populations further. By exposing target weeds to Sonar prior to harvest, viability of fragments may be reduced. This study was conducted to assess the effects of cutting and exposure to Sonar on subsequent growth of removed fragments and on the remaining cut, rooted plant.

Methods and Materials: Excised 15 cm apical shoots of *M. spicatum* were planted in UC mix supplemented with 5 g Osmocote (120 release formulation) in 8" by 12" by 5" deep plastic pans and submersed in 30 gal plastic barrels filled with well water. Barrels were maintained outdoors under 60% shade cloth and flushed daily for two weeks before application of Sonar 4AS. Treatments were replicated 4x and included: 0, 10, 20, and 60 ppbw fluridone for 21 days. Sonar was refreshed 14 days after the start of the exposures. At the beginning of the exposure, lengths of primary shoots and numbers of lateral shoots was recorded. After 21 days, all Sonar was removed by three successive flushing with will water, and subsequent daily flushing. During treatments, flushing was discontinued. Ten 5-node apical cuttings were removed from exposed, rooted plants on the following schedule: 14 days after beginning treatments (14 ABT), 2 days, 7 days and 21 days after the end of the 21-day treatment (i.e., 2 AET, 7 AET, 21 AET). Thus, those tips first removed had been on plants exposed to Sonar for 14 days; whereas the last tips removed had been on plants exposed to the full 21 days plus another 21 days without Sonar. Separate sets of plants received no Sonar and were not cut. After cutting the 5-node tips, they were weighed, and transferred to 1 l flasks containing 500 ml well water and placed in a growth chamber at 20 to 22C, under ca. 200 μmol m^{-2} sec^{-1} fluorescent lighting, LD 14: 10. Tips were observed weekly and numbers of new shoots, lengths and roots were recorded and well water was replaced. Rooted plants were harvested 45 days after the end of the 21 day exposure to Sonar. Treatments were started 8/26/96 and the final harvest was 11/12/96.

Results: Cut tips on all plants not exposed to Sonar and cut up to 7 days after the end of the 21 day exposures gained fresh weight over the subsequent 35 days (Fig. 1). The greatest inhibition of growth in the cut tips resulted from exposures to 20 or 60 ppb fluridone, regardless of the time of cutting up to 7 days after the end of the exposures. However, even the 10 ppbw concentration reduced fresh weight significantly in all cutting regimes except 21 days after the end of exposures. Only those tips cut 21 days after the end of the exposure had fresh weight similar to unexposed plants. For a given observation interval, the greatest reduction in growth of tips was observed on those cut 14 days after beginning the exposure and those cut only 2 days after fluridone had been removed. The lengths of the cut tips were also reduced in the plants exposed to 20 and 60 ppb fluridone in those removed 14 days after the beginning of the treatment and at the end of the 21 day exposure ("initial" Cut 7 AET). However, tips removed at 7 AET recovered to attain lengths similar to those in unexposed plants. Final biomass (d.wt.) of rooted plants is shown in Figure 3 from which it appears that the combination of cutting and 10 or 20 ppbw fluridone resulted in lower production than either treatment alone. The highest concentration, 60 ppbw, resulted in the most inhibition regardless of cutting regime, except for 21 AET. However, even the unexposed plants cut at 7 AET or 21 AET had lower final biomass compared to those cut earlier.

Although these data are preliminary, they suggest that the efficacy of Sonar, particularly at low rates, may be enhanced by cutting, presumably through stimulation of re-growth and more actively growing tips. In addition, the responses of the cut tips indicates that mechanical harvesting two to three weeks after beginning Sonar treatments may result is far less viable fragments than otherwise might be produced. This approach may be important where it is necessary to remove unwanted biomass without risking the spread of this week to uninfested areas.

Figure 1. *M. spicatum* excised shoot FW after 21 day exposure to Sonar (Treatment date was 8/26/98).

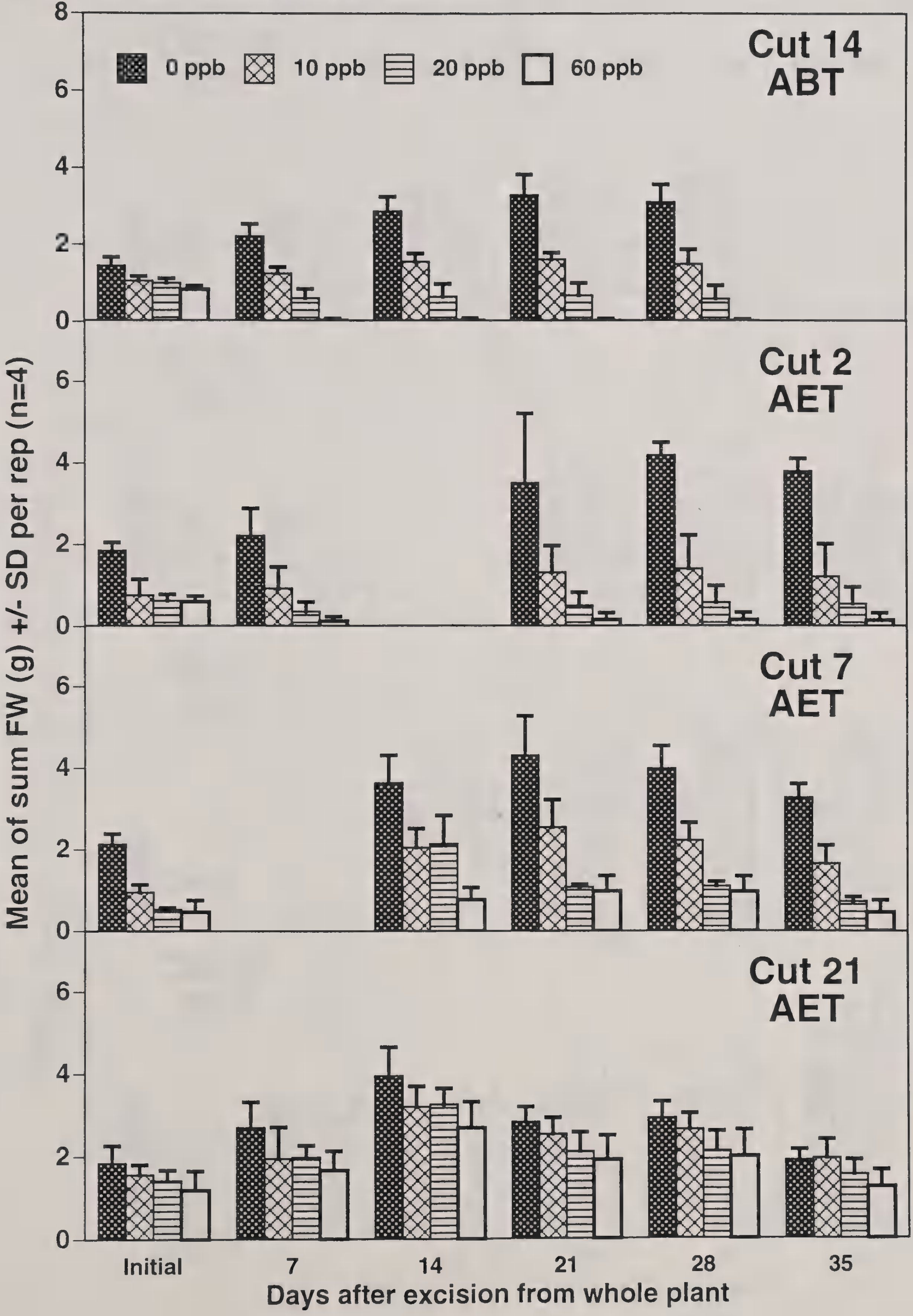


Figure 2. *M. spicatum* excised shoot re-growth after 21 day exposure to Sonar (Treatment date was 8/26/96)

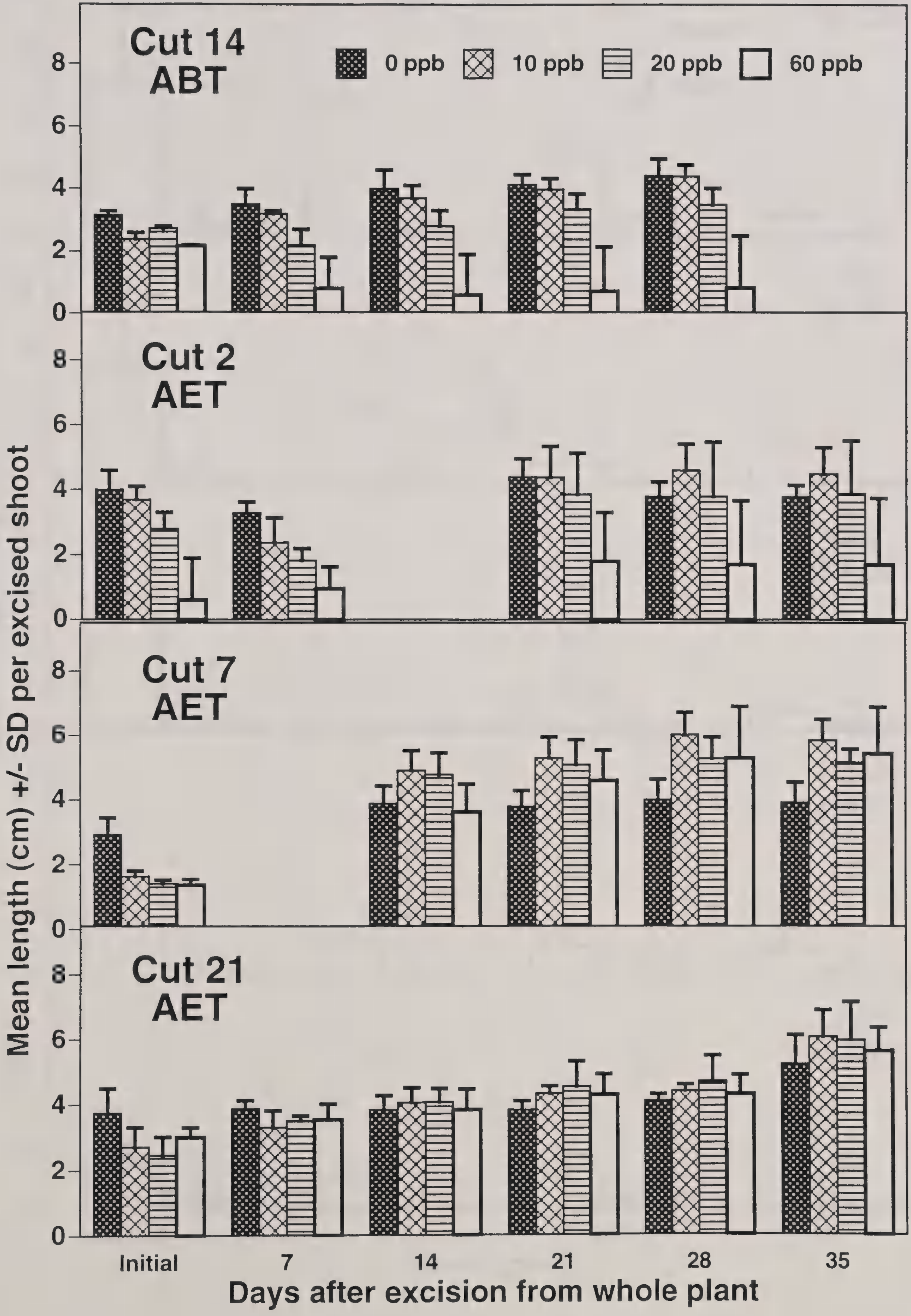
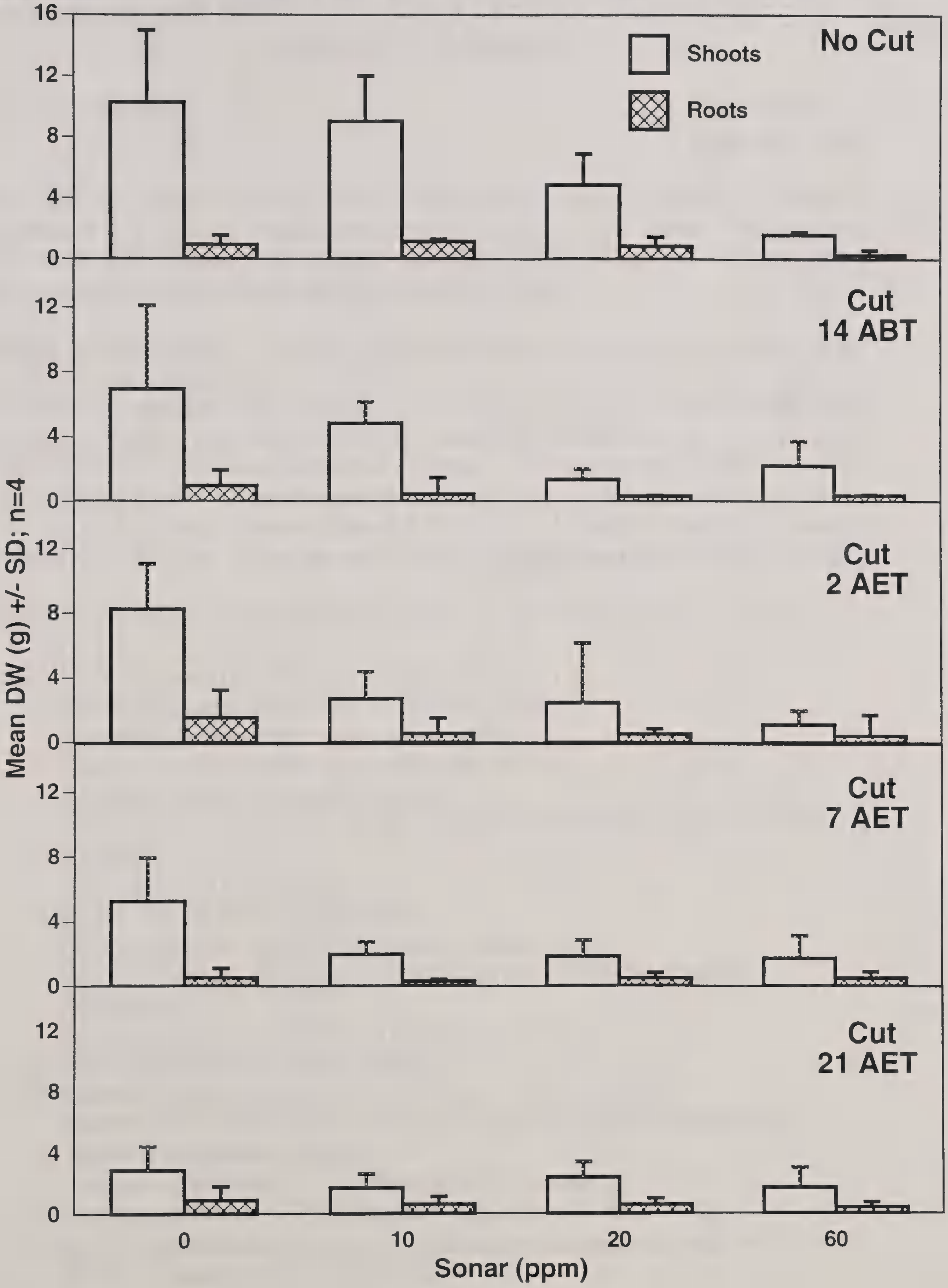


Figure 3. Effect of cutting and 21 day Sonar exposure to rooted M. spicatum (Treatment date was 8/26/96



UPTAKE AND TRANSLOCATON OF FLURIDONE BY *SCRIPUS* FROM WATER IN CLEAR LAKE AFTER APPLICATION FOR HYDRILLA CONTROL

Reporting scientists: Gee, Doreen
Anderson, Lars

Objective: To control hydrilla in Clear Lake in Lake County, California, California Department of Food and Agriculture applied fluridone to the water. The purpose of this study was to determine whether *Scripus* (tule) absorbed the fluridone and if it did, whether it was translocated throughout the plant.

Methods and Materials: Two sites where selected for this study--Red Bud Park and Rancheria. The Red Bud Park site was designated as the control site and thus was not treated with fluridone. The Rancheria site was treated with fluridone. There were three collection dates, pretreatment on 7/1/97, post-treatment 7/22/97, and final collection on 8/5/97. Fluridone was applied at a rate of 20 ppb on 7/8/97. At each site, 5 replicates were collected. The replicates consisted of 4 plants that were divided into top shoot, middle shoot, basal shoot, rhizomes, and flowers. After the plants were collected, they were stored at -20℃.

Fluridone was extracted from all the samples using the following protocol:

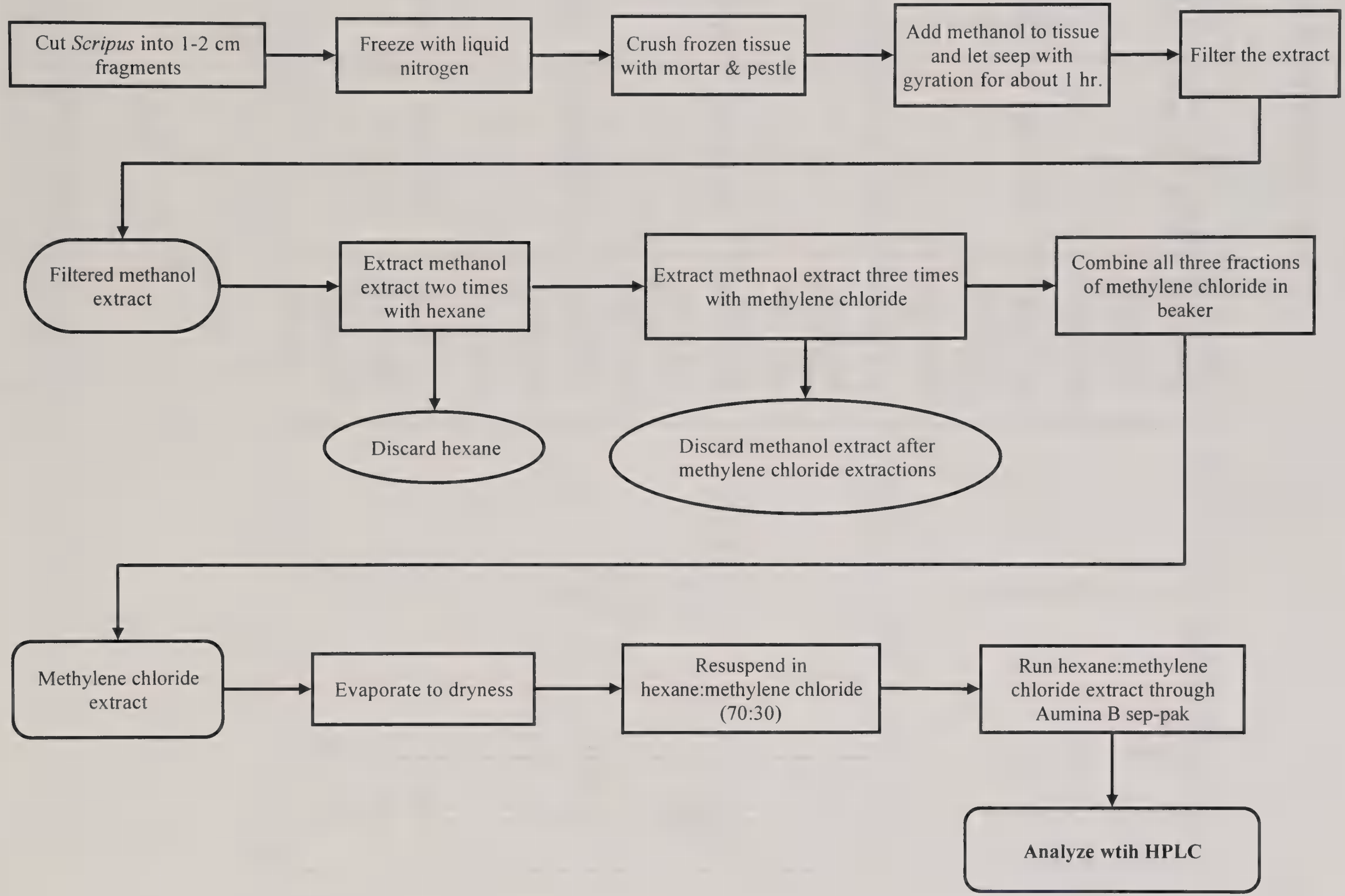
--Freeze up to 10 g of tissue with liquid nitrogen
--Break up frozen tissue with mortar and pestle
--Transfer to beaker and add 100 ml methanol
--Shake on rotary shaker for approximately 1 h
--Filter and collect methanolic extract
--Add to separatory funnel containing equal volume 5% sodium chloride solution
--Add equal volume hexane
--Shake vigorously until well mixed
--Collect aqueous phase and discard organic phase
--Return aqueous phase to separatory funnel and repeat hexane separation
--To aqueous phase add 40 ml methylene chloride
--Shake vigorously until well mixed
--Collect organic phase and filter through sodium sulfate
--Repeat methylene chloride step two additional times combining all methylene chloride filtrates
--Evaporate methylene chloride filtrates to dryness
--Resuspend in 5 ml 70:30 hexane:methylene chloride
--Apply resuspended sample onto alumina B sep-pak®primed with 10 ml 70:30 hexane:methylene chloride

--Rinse sample container 2 additional times with 5 ml 70:30 hexane:methylene chloride and apply to alumina B sep-pak®
--Wash sep-pak with 10 ml 70:30 hexane:methylene chloride
--Wash sep-pak with 5 ml 1% methanol
--Elute fluridone with 5 ml 1% methanol
--Evaporate methanol extract to dryness
--Resuspend in 250 -500 µl 70:30 methanol:water
--Store at 4°C

Each sample was analyzed for fluridone with high pressure liquid chromatography (HPLC) on a 10 µm µBondapak column under the following conditions: isocratic, 55:45 methanol:water, 1 ml/min. The retention time was approximately 25 min.

Results: The results are summarized in the following Table 1. Fluridone was not detected in the Red Bud Park samples nor in the Rancheria samples that were collected prior to treatment. Fluridone was detected in all Rancheria plant samples harvested post-treatment. All plant segments analyzed contained fluridone indicating that not only was fluridone taken up by*Scripus* but that it was also translocated throughout the plant.

TENTATIVE PROTOCOL FOR THE EXTRACTION AND ANALYSIS OF FLURIDONE FROM *SCRIPUS*



12/22/97

Table 1: Levels of fluridone in *Scripus* samples detected with HPLC

DATE	SITE	SEGMENTS	PPB (ng fluridone/ g tissue)	SD
7/1/97	Red Bud Park	Shoot Top	0	0
		Shoot Mid	0	0
		Shoot Basal	0	0
		Rhizomes	0	0
		Flowers	0	0
7/1/97	Rancheria	Shoot Top	0	0
		Shoot Mid	0	0
		Shoot Basal	0	0
		Rhizomes	0	0
		Flowers	0	0
7/22/97	Red Bud Park	Shoot Top	0	0
		Shoot Mid	0	0
		Shoot Basal	0	0
		Rhizomes	0	0
		Flowers	0	0
7/22/97	Rancheria	Shoot Top	52.5/3.5[1]	110/3.4
		Shoot Mid	3.2	4.5
		Shoot Basal	6.2	8.6
		Rhizomes	15.2	13.6
		Flowers	28.3	39
8/5/97	Red Bud Park	Shoot Top	0	0
		Shoot Mid	0	0
		Shoot Basal	0	0
		Rhizomes	0	0
		Flowers	0	0
8/5/97	Rancheria	Shoot Top	9.6	9.2
		Shoot Mid	4.7	5.2
		Shoot Basal	6.6	4.2
		Rhizomes	50.1	50
		Flowers	14	18.4

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[1] In one of the samples, the detected value was inordinately high compared to the other replicates. The first number (52.5 ± 110) is the mean of the replicates including this value while the second number (3.5 ±3.4) is the mean not including this value.

DOUBLE TIDAL-INJECTION OF KOMEEN FOR CONTROL *OF Egeria densa* IN SAND MOUND SLOUGH AND SEVEN MILE SLOUGH

Reporting Scientist:	Anderson, Lars
Associated Technicians:	Pirosko, Chris Duvall, Rob Holmberg, Debe

Objective: The development of effective, cost-efficient methods for management of *E. densa* is essential in order to implement state-mandated egeria control program for the Sacramento-San Joaquin Delta. This study is a follow up to prior work that employed submersed and floating direct-injection systems and predictable tidal flow to distribute herbicides.

Methods and Materials: A floating, multi-port injection manifold was used to apply Komeen on both in-coming and out-going tides at Sand Mound Slough and Seven Mile slough (See 1996 An. Report for diagram of apparatus). The entire system was placed perpendicular to the shoreline and covered ca. 200 linear feet. The manifold was connected to a 5 hp pump that delivered 150 gal. Komeen at 20 psi for approximately 3 hours beginning one hour after low, "slack" tide. After the first period of injection, the pump and manifold system was moved ca. 3,500 ft. "downstream". From this second point, another 150 gal. Komeen was injected on the outgoing tide for ca. 3 hours. Biomass was sampled using a 0.25sq.m quadrat before and 14 DAT. Pre-injection and post-injection water samples were taken in duplicate at designated stations with a submersible pump placed at 1/3 the depth from the surface and 1/3 the total depth from the bottom. Sampling intervals for Sand Mound Slough were: 3-3.5h, 7-7.5h, 9:45-10:45h and ca. 25 h post injection. For Seven Mile Slough, intervals were: 3-5.5j, 6.5-7.75h, 9-10h, and 24-25.5h. Intact shoot tips were collected for analysis of copper at pre- and 7h and 25 h posttreatment at Sand Mound Slough. Shoot tips were collected at the same time as all water sampling at Seven Mile Slough. Copper was analyzed via AA after acidification of water and after digestion of plant tissue with 4N HNO_3.

Results: At Sand Mound Slough, Cu levels reached just under 0.2 ppmw within 3 to 10 h post injection (Fig. 1). Little difference was seen in Cu levels in samples from the top and bottom 1/3 of the water column. By 24 h post-injection, levels of Cu had declined to nearly background. Between 3h and 7 h post-injection, copper had spread to most of the sampling stations, but at very low levels. Generally, the highest tissue-load of Cu (200 to 400 ppm) at 24 h was associated with the greatest decline in biomass 14 DAT (Fig. 2).

The pattern of Cu movement and dissipation was similar in Seven Mile Slough (Fig. 3), except that maximum Cu levels were ca. 1.0 ppmw. Also, there appeared to be less vertical mixing since top and bottom samples had significantly different levels of Cu at 7h and 9 h post-injection. This may be due to more rapid and unimpeded tidal flow in this slough compared to Sand Mound. At Sand Mound Slough, a tidal-flap gate closes on the incoming time

and this results in “pooling” of water at one end. At Seven Mile Slough, there seemed to be more “downstream” dissipation on the second, outgoing tidal injection than on the first, incoming injection period (Fig. 3). The reductions in biomass were more isolated to the mid-position stations, where, coincidentally, plant Cu levels were also the highest (Fig. 4).

Taken together, the dual-injection approach resulted in biomass reduction in ranges from 2,000 ft (Seven Mile Slough) to over 4,000 ft. (Sand Mound Slough). Given the width of the soughs (200 to 300 ft.), this resulted in reduction of biomass in from 15 to 25 acres. These data also are consistent with prior results showing that tissue levels in *E. densa* of 200 to 400 ppmw are sufficient to cause defoliation and collapse of stems within 14 days.

Figure 1. Total Cu in water column of Sand Mound Slough following Komeen applications by the floating injection manifold on 6/2/97

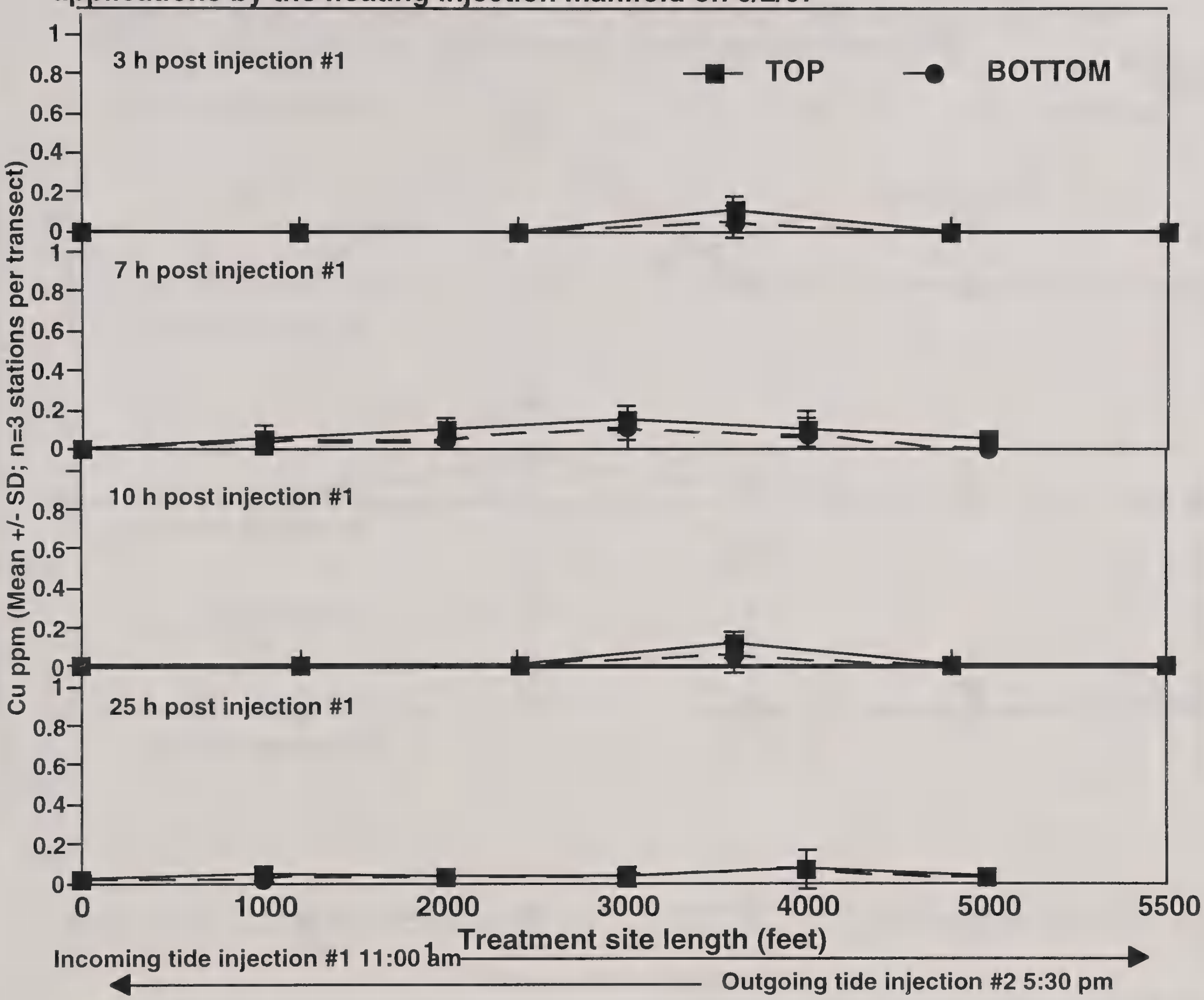


Figure 2. Egeria densa dry weight in Sand Mound Slough and Cu level in shoot tips 24 hours post injection of Komeen by the floating manifold on 6/2/97

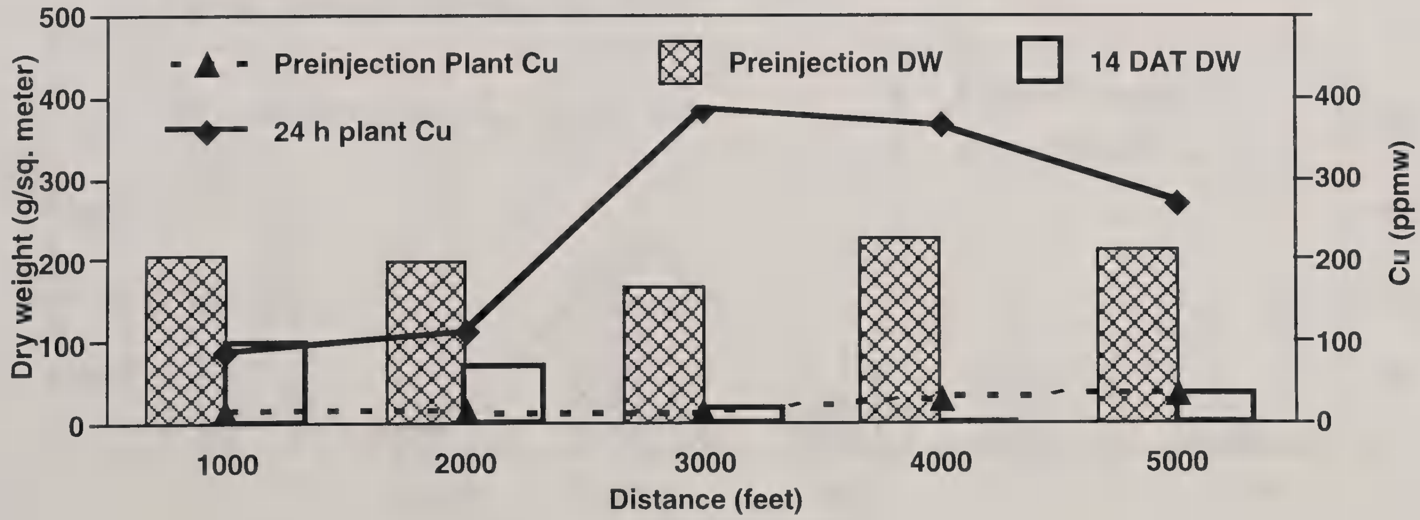


Figure 3. Total Cu in water column of 7 Mile Slough (Owl Harbor) following Komeen applications by the floating injection manifold on 7/2/97

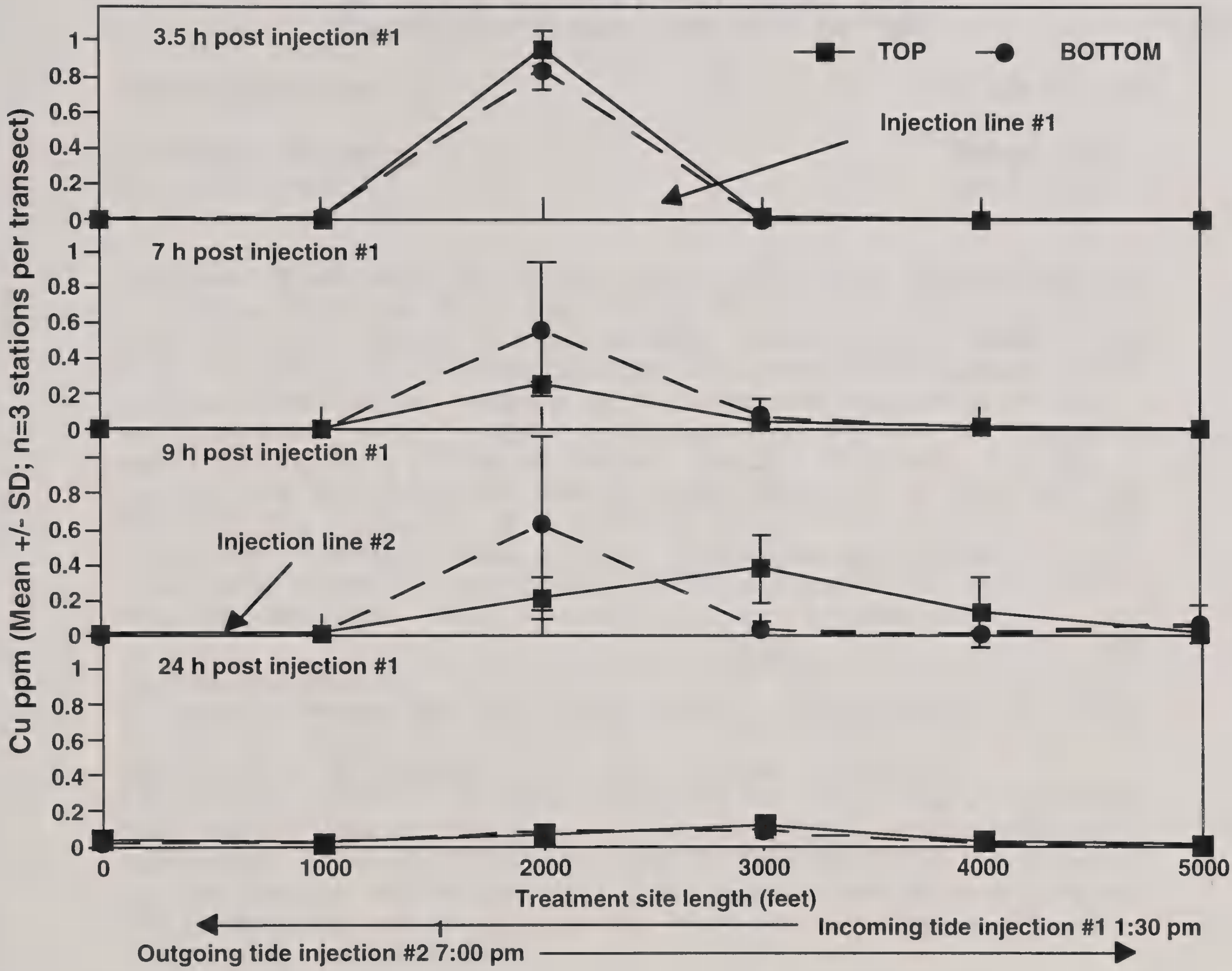
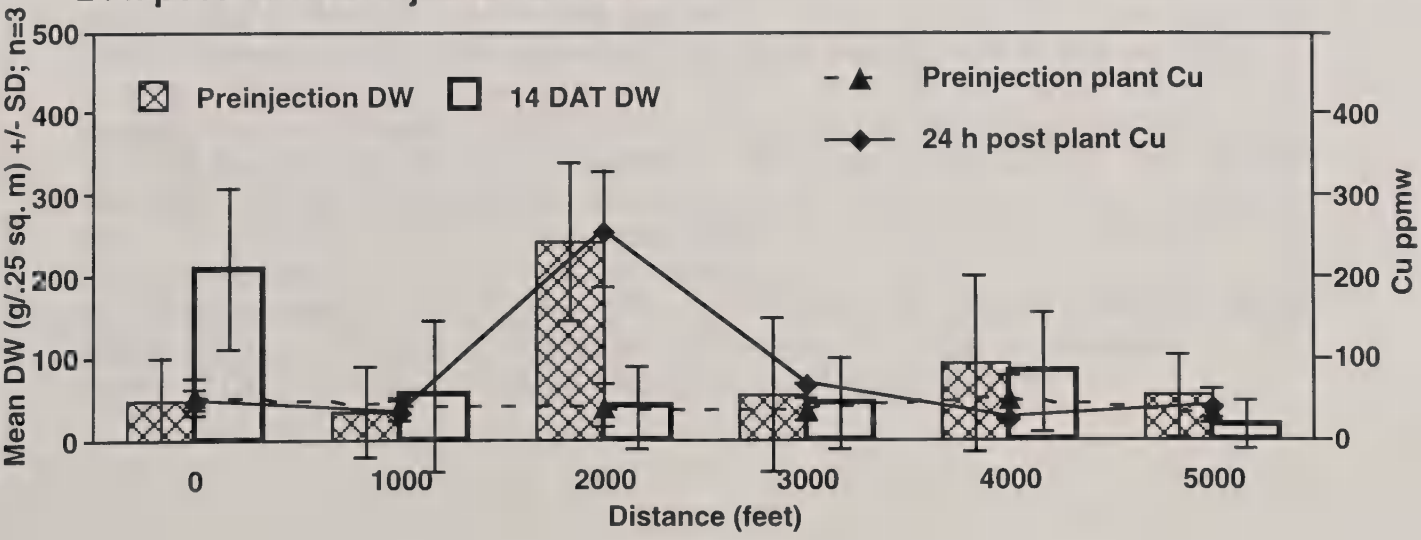


Figure 4. Egeria densa DW in 7 Mile Sl and Cu level in shoot tips 24 h post Komeen injection on 7/2/97



EFFICACY OF KOMEEN ON *Egeria densa* IN THREE SITES AT DISCOVERY BAY (SACRAMENTO-SAN JOAQUIN DELTA)

Reporting Scientist:	Anderson, Lars
Associated Technician:	Pirosko, Chris
Graduate Student:	Duvall, Rob

Objective: Flowing water conditions in the Sacramento-San Joaquin Delta are a significant challenge use of conventional aquatic herbicides since dilution rates are high and contact time with the target plant is extremely limited. This study was done to determine the dissipation of Cu and it effectiveness on egeria growing in three different sites that represent high and moderate to low water-exchange areas. Indian Slough is a highly exposed, high-flow tidal area with egeria growing along a fringe of shoreline from 2 to 10 m wide. Lido Bay is "pocket" type embayment that has moderate exchange of water and the Discovery Bay Marina site is a protected area at the "dead" end of an embayment housing hundreds of boats. It undergoes tidal flushing but at a lower exchange rate than the other sites. Plants at Discovery Bay Marina form a long, very narrow (ca. 1 to 1.3 m wide) band adjacent to the shoreline.

Methods and Materials: Pre-application biomass samples were taken using a 0.25 sq.m qudrat and fixed water sampling stations were established at each of the three sites. Komeen was applied by boat with weighted, flexible hoses and injected 12" to 18" underwater to produce a nominal concentration of 0.5 ppmw (at high tide). The Indian Slough site was 225 m long; Lido Bay was 80 m long and Discovery Bay Marina site was 60 m long. Water sampling was conducted at 1/3 depth to the surface and 1/3 total depth from the bottom in duplicate using a submersible pump. Intact egeria shoot tips were collected 24h posttratment and analyzed for Cu level after digestion in 4N HNO_3. Biomass was sampled 16 DAT.

Results: Copper levels ranged from 0.2 ppmw to 0.4 ppmw 4 h postreatment in Indian Slough, but declined to ca. 0.1 ppmw 9 h posttreatment (Fig. 1). By 25 h posttreatment, Cu was at or near background levels. Little Cu was found outside the immediate area of the treated plot (e.g. stations #6, #7) suggesting that a general dilution with incoming tidal water cause most of the reduction in copper. Figure 2 show that the Lido Bay site had ca. 8.0 ppmw shortly after application and sustained levels at or above 0.2 ppmw for at least 8.5 h. Levels were at background 24 h posttreatment. Although the Discovery Bay Marine site had only ca. 0.3 ppmw Cu at the beginning, it still had 0.075 to 0.2 ppmw 8.5 h posttreatment (Fig. 3), indicating that less tidal exchange occurred there. All applications significantly reduced biomass, particularly where copper levels in the plant tissues were highest (Fig. 4). For example, plants at station #8 in Indian Slough showed an increase in biomass and also no increase in the levels of Cu in tissues compared to pre-treatment levels (Fig. 4).

Figure 1. Total copper in water column of Indian Slough following Komeen application on 9/16/97

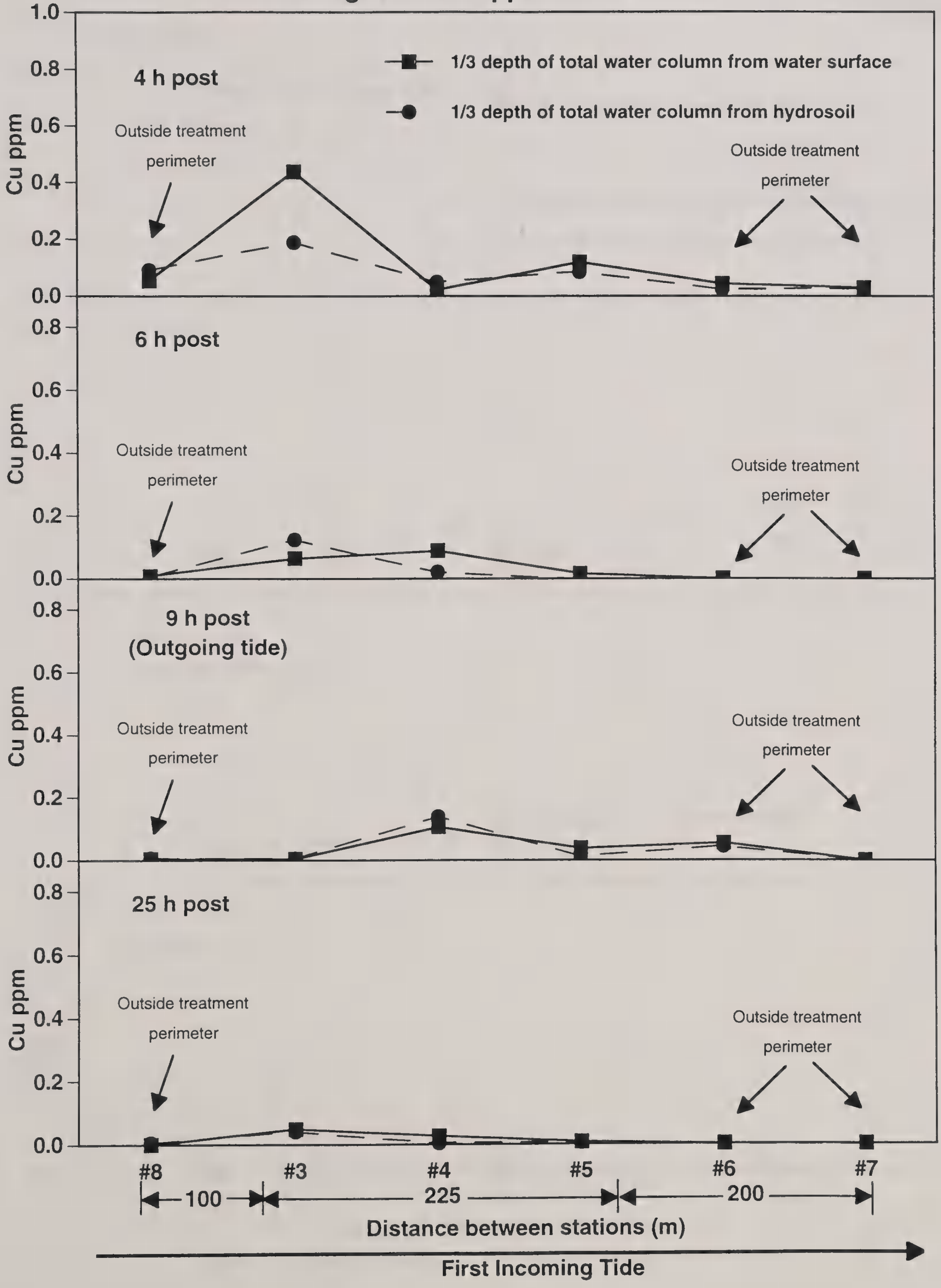


Figure 2. Total copper in water column of Lido Bay following Komeen application on 9/16/97

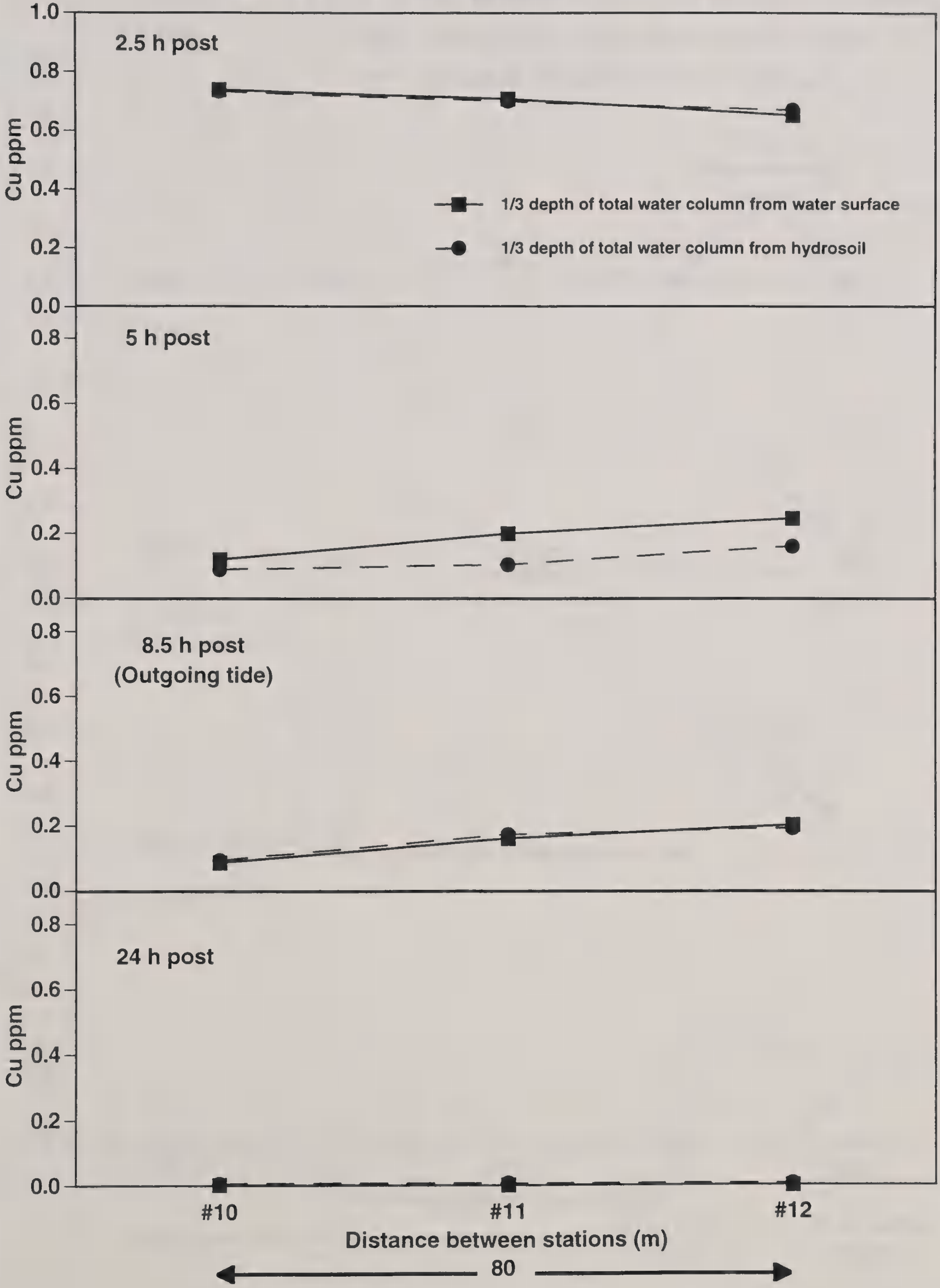


Figure 3. Total copper in water column of Discovery Bay Marina following Komeen application on 9/16/97

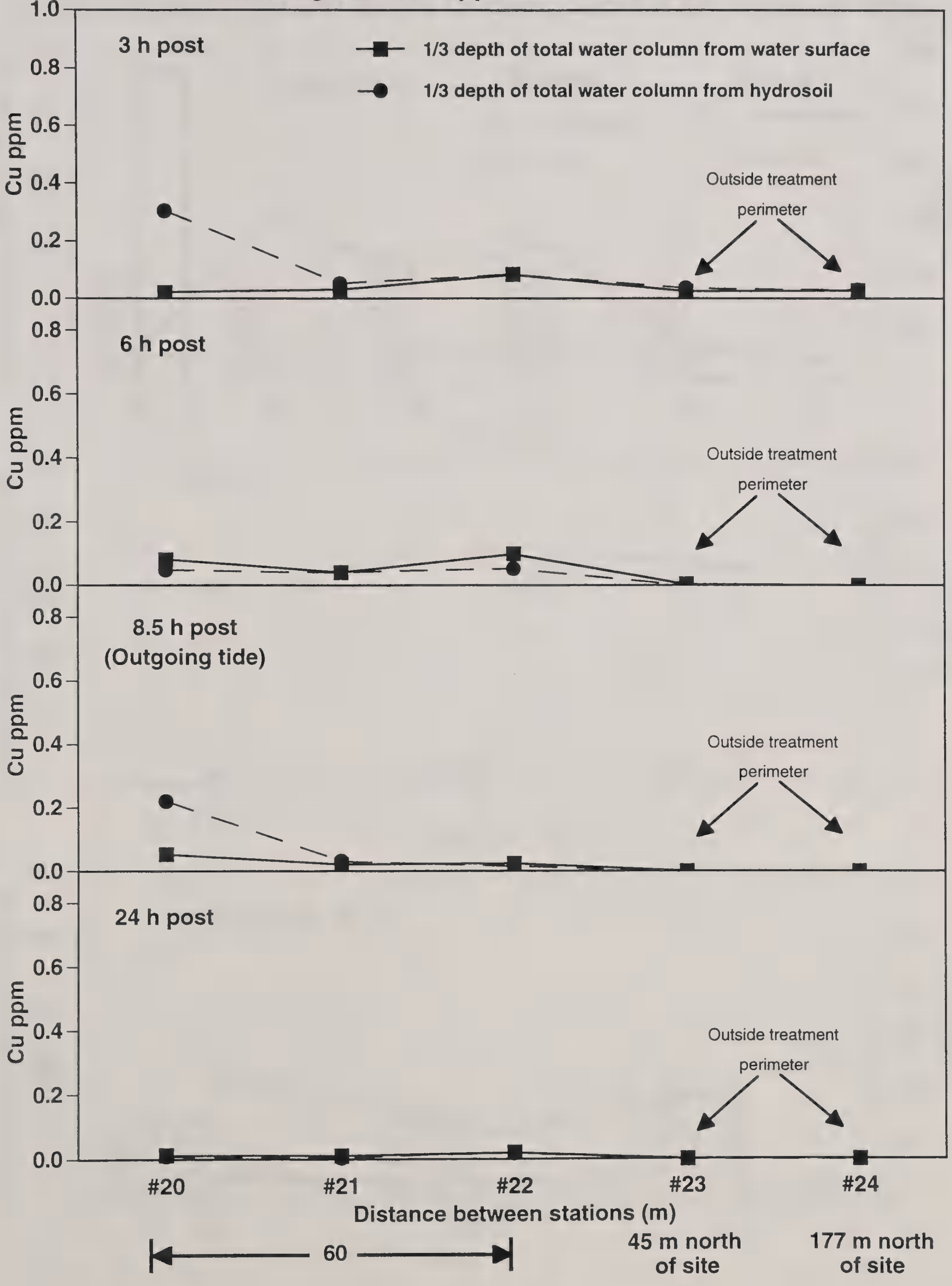
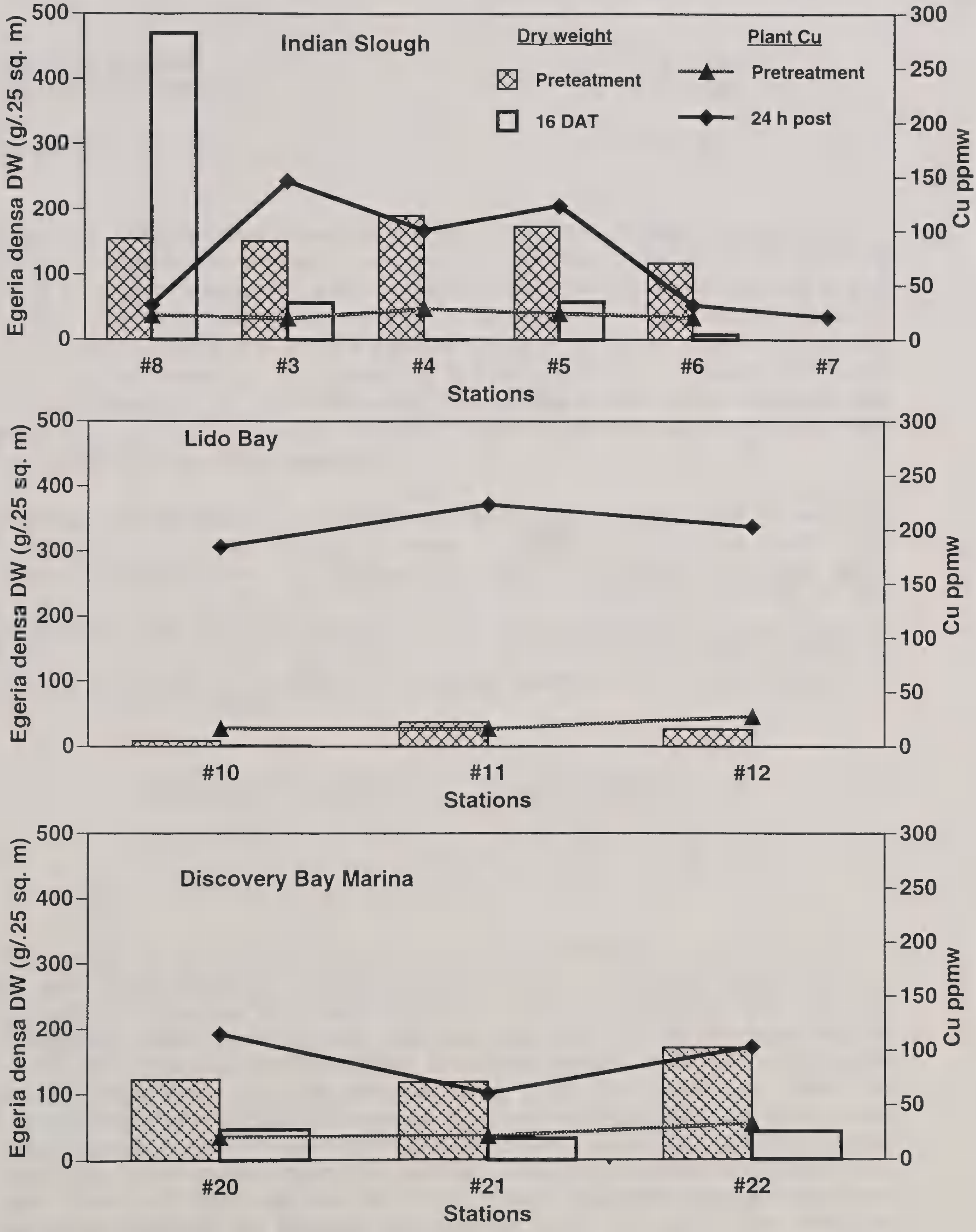


Figure 4. *Egeria densa* DW and Cu levels in shoot tips of Indian Slough, Lido Bay, and Discovery Bay Marina following Komeen application on 9/16/97



INFLUENCE OF DILUTE ACETIC ACID TREATMENTS ON *HYDRILLA* TUBERS IN THE OREGON HOUSE CANAL, YUBA COUNTY, CALIFORNIA

Reporting Scientist D. F. Spencer
Associated Technician G. G. Ksander

Cooperator N. Dechoretz

Objective: Previous work (Spencer and Ksander 1995, Aquatic Botany 52: 107-119) indicates that in laboratory and greenhouse trials dilute solutions (0.1 to 5 %) of the natural sediment component, acetic acid, greatly reduced sprouting and survival of *Hydrilla* tubers. The purpose of this work was to answer the following questions: I) Does acetic acid kill *Hydrilla* tubers in an undisturbed canal setting (as it does in smaller scale studies)? 2) Is there a difference between a 2.5 and 5% concentration? 3) Does perforating the sediment improve the treatment by enhancing penetration into the sediment? 4) Do important sediment properties change as a result of this treatment?

Materials and Methods: We conducted the following experiment to evaluate acetic acid treatments for *Hydrilla* tubers in undisturbed canal sediment. The experiment follows a two-way analysis of variance design with the two treatments being acetic acid (0, 2.5, and 5%) and sediment perforation (perforated vs. not perforated). The sediment perforation was accomplished by forming 15-cm (6 inches) deep holes into the sediment prior to treatment with acetic acid. This allowed evaluation of the effect of improved sediment penetration. Below is a diagram of the treatments.

Sediment Perforation	Acetic Acid (%)		
	0	2.5	5
No	10	10	10
Yes	10	10	10

There were 10 replications (plots) per treatment combination. Each plot (1 m x 1 m) was surrounded by sandbags. The sediment perforation treatment was applied with a potato fork (tines were 15 cm long (6 inches)) and, then the appropriate acetic acid treatment. Each plot was flooded to an average depth of 10 cm (4 inches) with the appropriate acetic acid treatment or well water as the control. Plots were randomly assigned to a particular treatment combination using PROC PLAN in SAS. Two weeks later, we removed three cores from each plot. One core was processed to remove the tubers present by washing it over a 2-mm mesh size metal screen. The number of tubers present was counted. One tuber from each plot was evaluated for relative electrolyte leakage using the procedure described by Spencer and Ksander (l997, J. Aquatic Plant Manage. 35: 25-30). Previous work indicated that tubers treated with acetic acid displayed

increased electrolyte leakage. The number of tubers per plot was used as the response variable in the two-way analysis of variance (ANOVA). Relative electrolyte leakage was used as the dependent variable in logistic regression against perforation treatment and acetic acid concentration. A second core was placed in a container and then in an outside tank filled with water. We monitored tuber sprouting over time for these cores. After three months we processed the cores to determine the total number of non-sprouted tubers remaining. The proportion of sprouted tubers was used as the response variable in logistic regression against perforation treatment and acetic acid concentration. The third core was processed for sediment particle size distribution, available phosphorus, exchangeable ammonium, nitrates, and potassium by the DANR analytical laboratory. Organic content was estimated by loss on ignition at 550 C. We conducted this research in an irrigation canal near Oregon House, Yuba County, California. On March 3, 1998, we retrieved 10 cores from the canal. Two cores were from each treatment and 6 were from areas outside the test plots. We used tubers from these cores in a sprouting test as described above.

Results: Relative electrolyte leakage (REL) increased for tubers from plots treated with acetic acid ($P < 0.001$, Figure 1). Tubers from treated plots that were perforated did not display increased REL at 2.5% acetic acid, but did at 5% (Figure 1). This is reflected by the significant interaction term in the analysis of variance. It indicates that perforating the sediment may have reduced acetic acid effects by enhancing the rate at which the added acetic acid percolated through the soil thus reducing contact time with tubers. Then number of tubers per plot did not differ among treatment combinations ($P > 0.05$, ANOVA). This is not unexpected since the tubers were collected just 2 weeks after the initial treatment and sediment conditions (low temperatures, not flooded) may not have been conducive to rapid decay of damaged tubers. The proportion of tubers sprouting after 21 days in the growth chamber decreased with exposure to acetic acid ($P < 0.0001$, logistic regression, Figure 2). As with the REL results, some tubers survived the 2.5% exposure if they were from plots with perforated sediments. Results form the cores monitored for sprouting over a 6-month period are shown in Figures 3 and 4. No tubers sprouted in the cores from the non-perforated plots that were treated with either 2.5 or 5% acetic acid, while the cores from control plots had 27 sprouted tubers. A similar response was observed for the cores from perforated plots, except that 4 tubers sprouted in the perforated plots treated with 2.5% acetic acid and 38 sprouted from control cores. This agrees with the other results that indicate that perforating the sediment actually lead to decreased affect of the acetic acid treatments. The acetic acid treatments did not alter the measured sediment characteristics (Table 1). Some other useful characteristics of the canal are presented in Table 2. Results of the sprouting tests for the tubers collected on March 3, 1998 indicated that none of the tubers from the test area, including those from control plots (20 in all) sprouted after 21 days, while those from outside the test area has 100% sprouting (27 total). At the time of treatment we noted that the control plots (3, 7) showed some evidence that the acetic acid from adjacent plots (5%, 2.5%) had leaked into these plots. This may explain the lack of sprouting for tubers from these plots.

Table 1. Sediment characteristics for Oregon House Canal plots. Values are based on 6 samples. Samples from perforated and non-perforated plots have been combined. Analysis of variance did not indicate significant differences due to acetic acid treatment.

Parameter	Acetic Acid (%)	Mean	Standard Error
Bray Phosporus (ppm)	0	5.60	1.14
	2.5	4.75	0.67
	5	3.55	0.31
NH_4-N (ppm)	0	48.72	26.75
	2.5	47.65	23.88
	5	20.38	4.64
NO_3-N (ppm)	0	3.27	0.92
	2.5	3.67	1.03
	5	2.05	0.39
K (ppm)	0	99.33	19.85
	2.5	86.33	6.48
	5	75.17	2.63
Organic Matter (%)	0	13.92	1.50
	2.5	14.20	1.40
	5	11.57	0.51

Table 2. Additional characteristics of the Oregon House Canal at the time this experiment was conducted.

Parameter	Mean	Standard Deviation	Number of Samples
Sediment Moisture (%)	41	5.8	9
% Sand	22.6	13.3	18
% Silt	58.4	13.4	18
% Clay	20	3.0	18
Width (m)	1.04	0.22	188

Figure 1

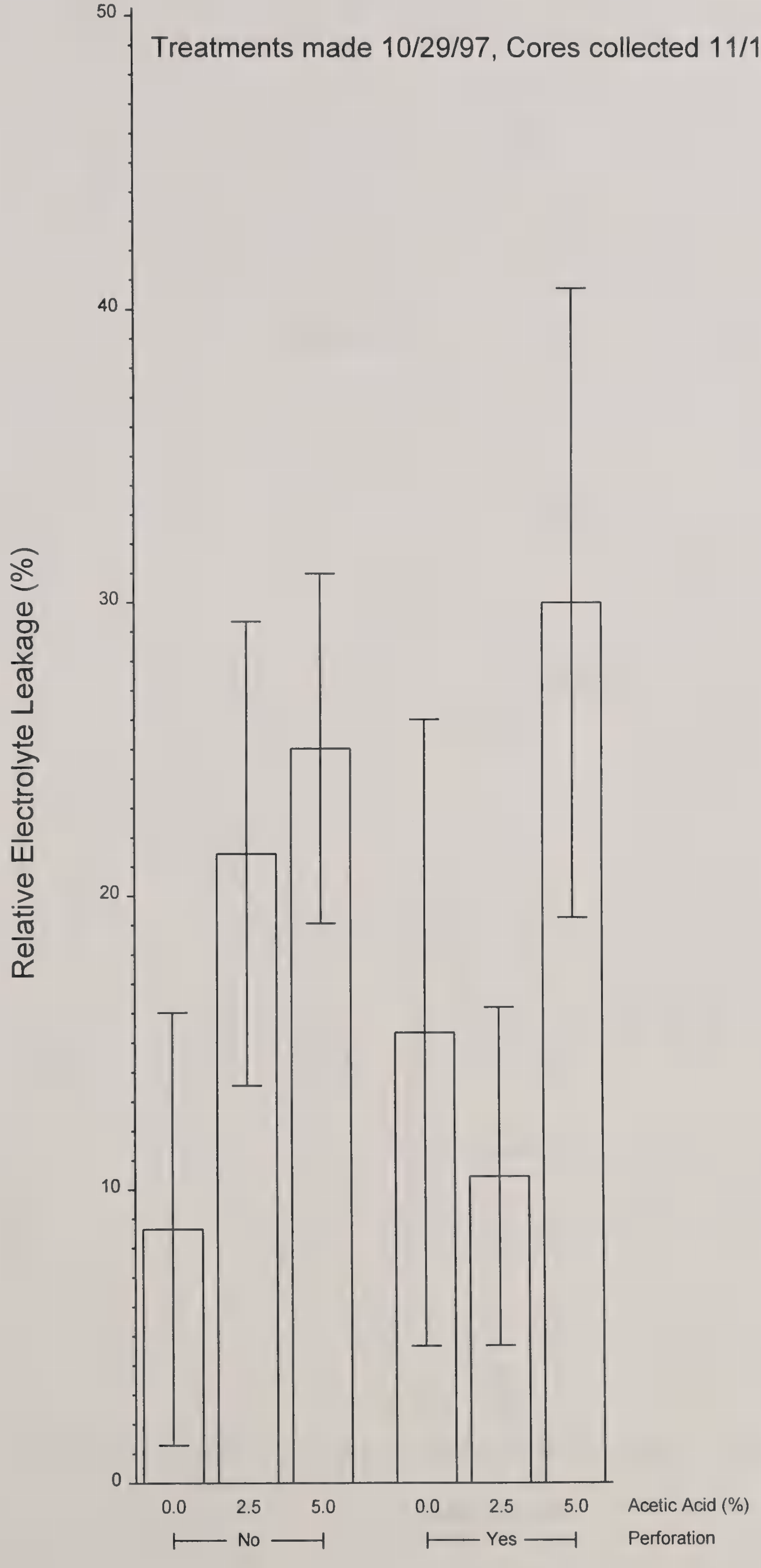
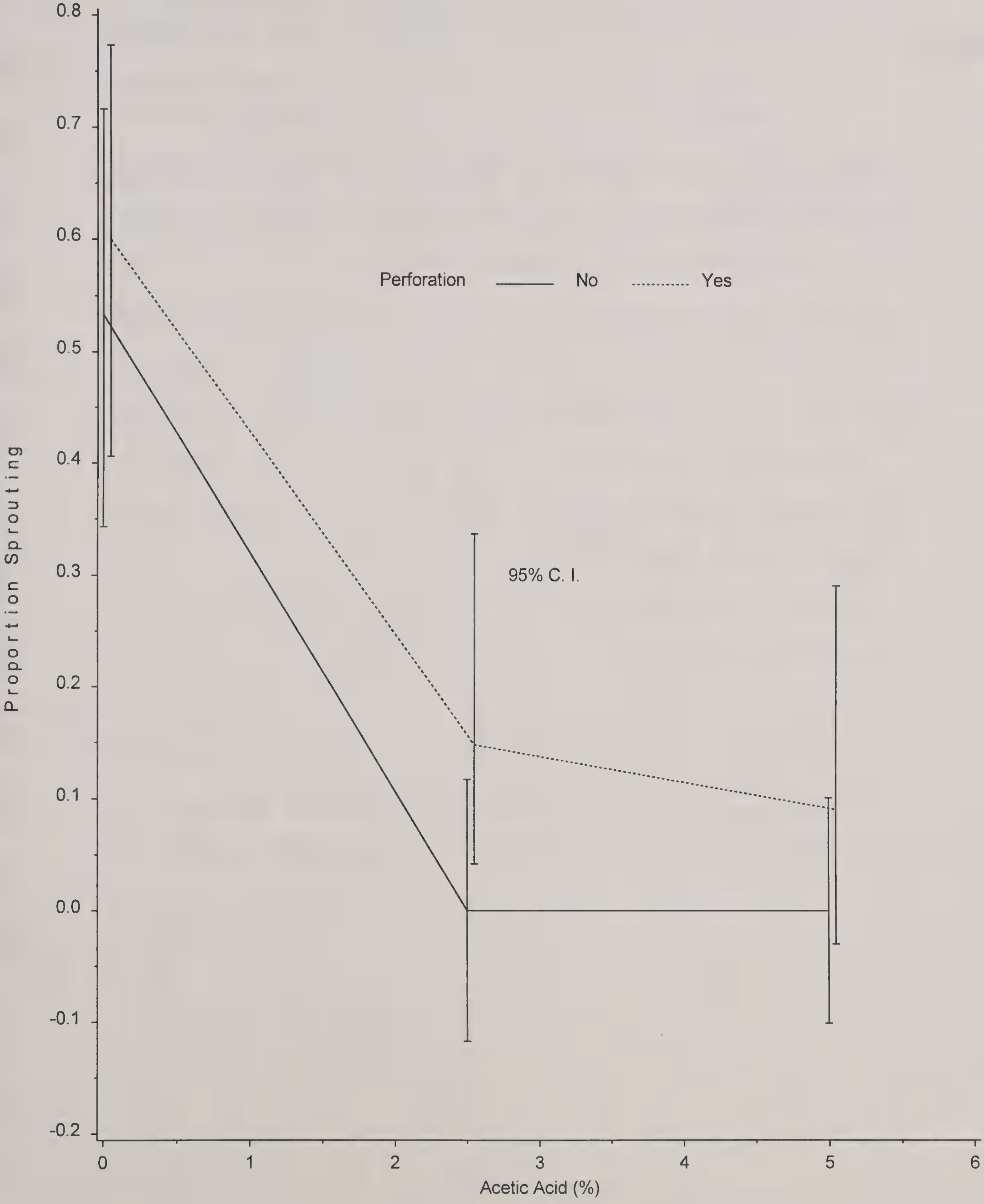

Treatments made 10/29/97, Cores collected 11/12/97
Relative Electrolyte Leakage (%)
50
40
30
20
10
0
0.0
2.5
5.0
0.0
2.5
5.0
Acetic Acid (%)
No
Yes
Perforation

Figure 2


Perforation
No
Yes
95% C. I.
Proportion Sprouting
Acetic Acid (%)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
0
1
2
3
4
5
6

INFLUENCE OF SEDIMENTATION ON HORNED PONDWEED (*ZANNICHELLIA PALUSTRIS*) EMERGENCE FROM FALL RIVER SEDIMENTS

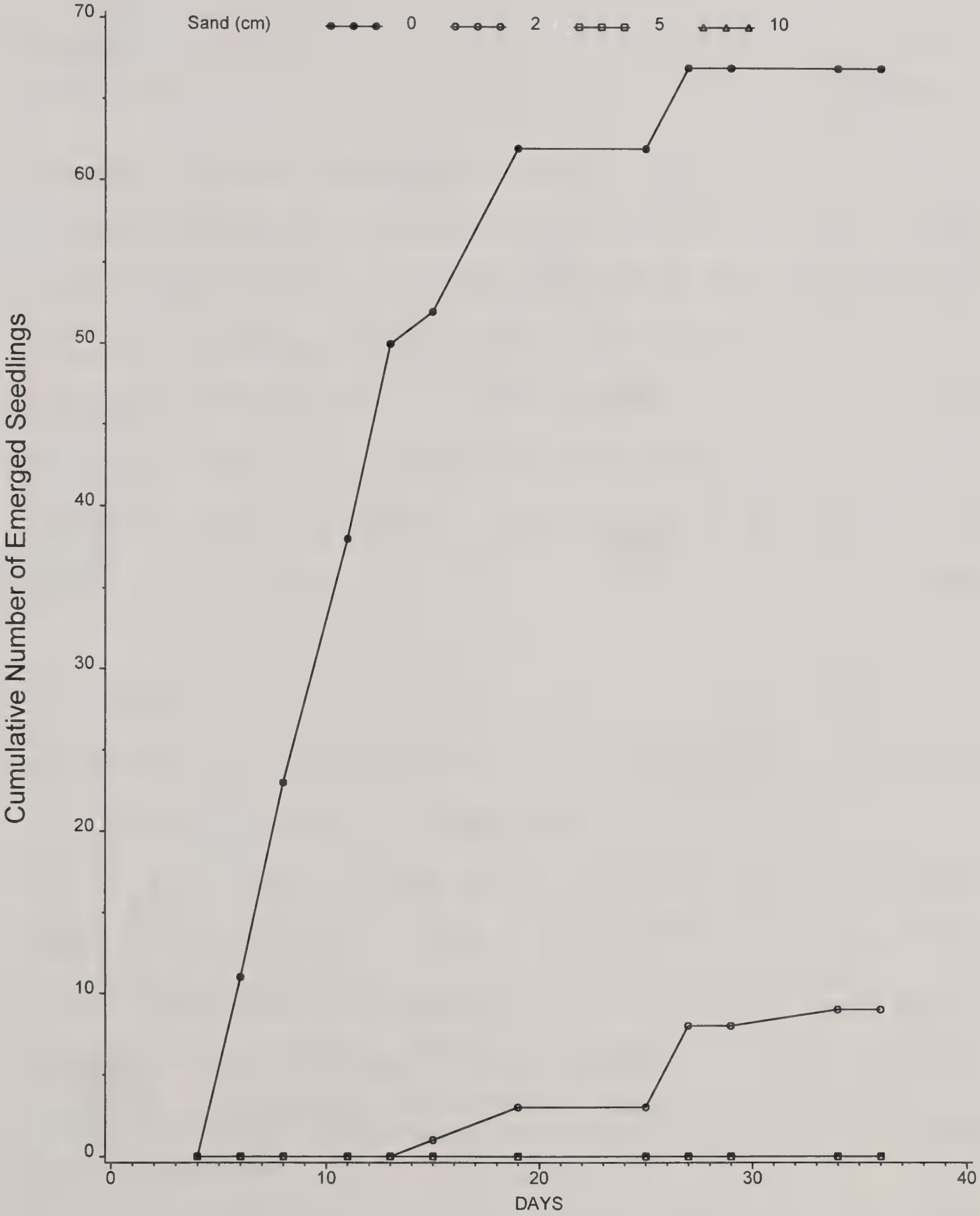
Reporting Scientist	D. F. Spencer
Associated Technician	G. G. Ksander

Objective: The aquatic plant community of Fall River, California has declined in areas where sandy sediments have accumulated. Previous greenhouse experiments indicated that seeds of *Zannichellia palustris* (horned pondweed) did not emerge from the sediment when buried under a few cm of sand. This experiment was performed to gain additional insight into this phenomenon.

Materials and Methods: On February 25, 1998 we collected sediment from a site just above the Whipple Bridge. Previous work indicated that *Zannichellia palustris* seeds were present in this area. The sediment was returned to Davis, and enough of it, to yield a depth of 1 cm, was placed in the bottom of 1-liter clear plastic pots (10.5 cm in diameter). There were 80 pots in all. In 20 pots, the sediment was covered with an additional 2 cm of sand. Twenty pots had 5 cm of sand added, and 20 pots had 10 cm of sand added. Twenty pots received no sand. Thus, this experiment was designed to measure germination of seeds collected from Fall River under various simulated sedimentation regimes. An important difference between this and prior experiments was that the seeds in this experiment had experienced ambient sediment temperatures since their formation and deposition into the sediments, during the prior summer, i.e., 1997. The experiment was set up within 24 h of sediment collection and the sediments were maintained in a sealed plastic bag at 4 ^{O}C during this brief interval. The pots were placed on a bench in the greenhouse (18-22 ^{O}C). The pots were checked every 2 to 3 days for presence of emerged seedlings.

Results: The graph below shows the cumulative germination for *Zannichellia palustris* in the pots. It is clear that more than 2 cm of sand on top of the sediment inhibited germination and emergence of *Zannichellia palustris*. These results agree with those from earlier experiments and indicate that sediment accumulation in an area of Fall River inhabited by *Zannichellia palustris* would lead to reduced germination and emergence of seedlings from the sediment.

Zannichellia palustris



PRELIMINARY STUDY OF AQUATIC INVERTEBRATES ASSOCIATED WITH THREE SPECIES OF AQUATIC PLANTS IN FALL RIVER, SHASTA COUNTY, CALIFORNIA.

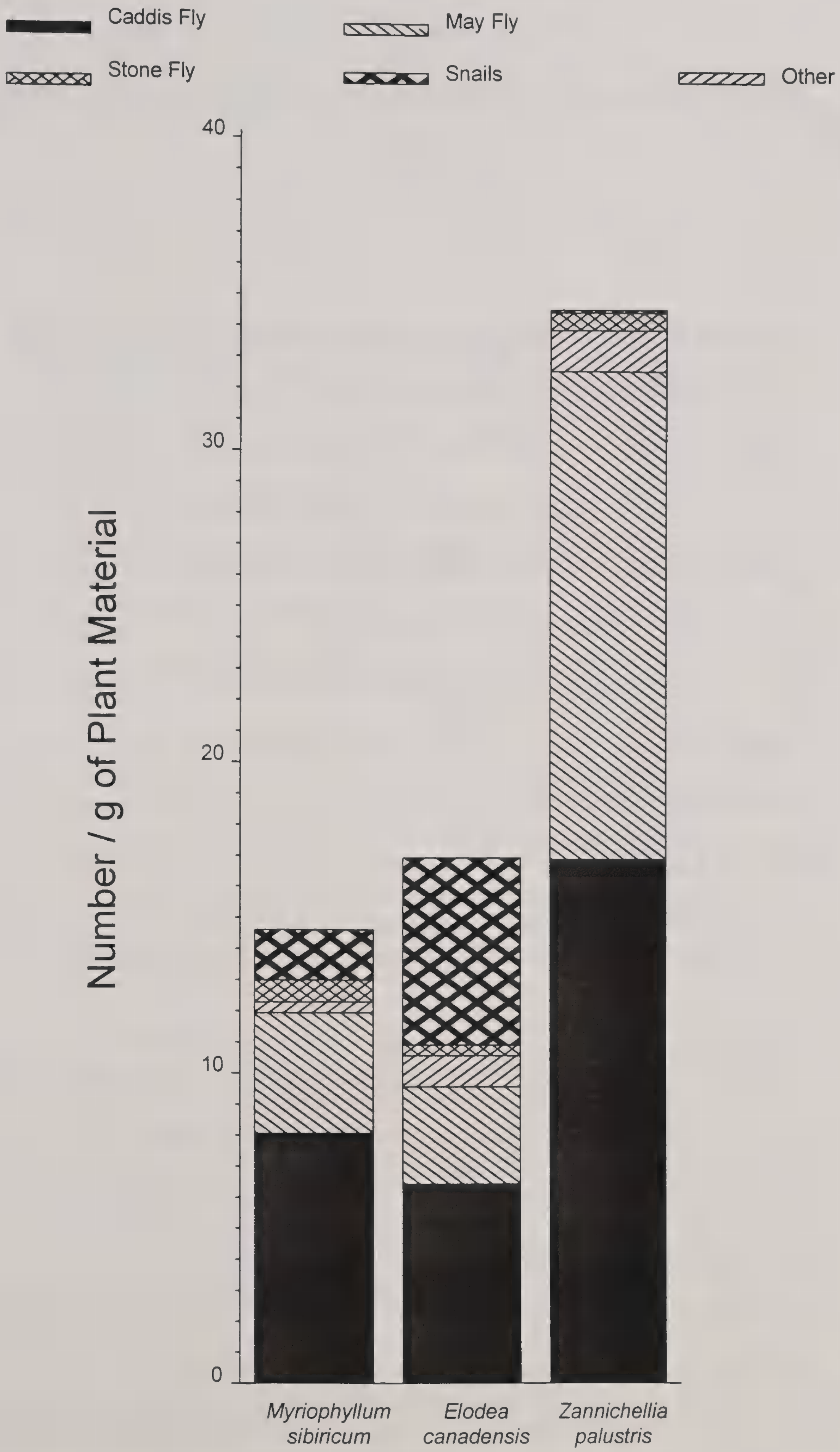
Reporting Scientist: D. F. Spencer
Associated Technician: G. G. Ksander

Objective: Eurasian watermilfoil *(Myriophyllum spicatum* L.*)* is an important introduced aquatic weed in North America, where it is found from Florida to Quebec in the east, and California to British Columbia in the west. Its strong competitive abilities allow it to displace native species, and its abundant growth impedes water use for agricultural, environmental, and recreational purposes. Current management techniques include mechanical and chemical methods. Recently, a native weevil *Euhrychiopsis lecontei* (Dietz), found in the northern and eastern states in the U. S. and associated with the native northern watermilfoil (*M. sibiricum*), has been suggested as a possible biological control for Eurasian watermilfoil. Eurasian watermilfoil occurs in California, but *E. lecontei* has not been reported from the state. The purpose of this study was to sample the aquatic invertebrates associated with *M. sibiricum* and other submersed plants in Fall River to determine if *E. lecontei* might be present. If *E. lecontei* occurs naturally in the state, then conducting bio-control experiments with it, would be less problematic.

Materials and Methods: Fall River is located in northern California in the northeastern corner of Shasta County about 97 km northeast of Redding. Fall River is a moderately sized, slow flowing, meandering meadow stream. It varies in width from 15 m near its headwater to 92 m in the lower reaches; depth ranges from approximately 0.6 m to an estimated 6 m in the deeper pools. On May 25, 1998, we used a weed rake to obtain samples of *M. sibiricum*, *Zannichellia palustris*, and *Elodea canadensis* from the river. *M. sibiricum* was collected at a site just below Fletcher's Bridge and *E. canadensis* and *Z. palustris* were collected at the Spring Creek Bridge. The plant samples were quickly placed in sealed plastic bags. Within 3 hours of collection, the invertebrates in each samples were separated from the plant material, using white plastic tubs and forceps. Invertebrates were preserved in 70% methyl alcohol. Upon return to the laboratory invertebrate samples were examined under a dissecting microscope. Insects were sorted to family. All snails were lumped together. The number of each category of invertebrates was counted. The plant material was dried for 48 h at 80 C. The number of invertebrates per gram dry weight of plant tissue was calculated.

Results: Immature stages of caddisflies, mayflies, and stoneflies were the most abundant invertebrates associated with the plant samples (Figure 1). Snails were found in the *M. sibiricum* and *E. canadensis* samples, but not the *Z. palustris*

samples. Interestingly, *Z. palustris* supported nearly twice as many invertebrates as did either of the other two species. The abundance of caddis flies or mayflies alone, on *Z. palustris* was equal to the total numbers of invertebrates on the other two species. Unfortunately, no weevils (*E. lecontei*) were observed on *M. sibiricum* on this sample date. However, these limited samples need to be increased by collecting additional samples at different times of the year.


Caddis Fly
May Fly
Stone Fly
Snails
Other
Number / g of Plant Material
0
10
20
30
40
Myriophyllum sibiricum
Elodea canadensis
Zannichellia palustris

USING VIDEOTAPED TRANSECTS TO ESTIMATE SUBMERSED PLANT ABUNDANCE

Reporting Scientist	D. F. Spencer
Associated Technician	G. G. Ksander

Objective: Assessing aquatic plant abundance and the effectiveness of various management techniques, often requires quantitative data in the form of plant coverage, distribution, and biomass. Several methods including line intercept-transects, aerial photography, and direct biomass sampling have been used to estimate submersed macrophyte abundance. Direct methods like biomass sampling, however, may be costly, labor intensive, and may be unsuitable when large areas must be sampled . Remote sensing techniques utilizing color and infrared aerial photography have been used successfully for emergent and floating aquatic macrophytes but have not been extremely successful for determining the abundance of submersed vegetation. We modified line transect procedures by using a glass-bottomed underwater viewer in conjunction with a hand-held light weight video camera to make videotapes of transects. We report here our experiences with the modified technique

Materials and Methods: This work was conducted in Fall River which is located in northern California in the northeastern corner of Shasta County about 97 km northeast of Redding. Fall River is a moderately sized, slow flowing, meandering meadow stream. It varies in width from 15 m near its headwater to 92 m in the lower reaches; depth ranges from approximately 0.6 m to an estimated 6 m in the deeper pools.. On July 23 and 24, 1996, we performed transects across the river at 56 locations in the portion of the upstream of Island Road Bridge. The locations of the transects were picked at random as we proceeded upstream in this 8 km section of the river.

Each transect consisted of the following procedure. An underwater viewing device was lowered into the water (only a few cm) on the sunny side of the boat. The viewer was shaped like an Erlenmeyer flask. It was 59 cm tall and had a 17-cm diameter glass bottom. An 8 mm video camera (Sony Model CCD-TRV1 1) was held above the viewer and the river bottom was videotaped as the boat was directed across the river. The video camera recorded the time, including seconds, directly on the videotape. Verbal comments on the aquatic plants present as well as other pertinent information were also recorded on the tape. We recorded the starting and ending location using a Garman Model 75 GPS unit, operating on version 2.32 software. This procedure was repeated on July 22 and 23, 1997, when we obtained data from 53 transects. For these transects we used a boat equipped with a 4 horsepower outboard engine and used the slowest possible speed given the engine size. Since engine speed may not be constant we attempted to measure its variation in the following manner. In

1997 we estimated the length of each transect using a Bushnell Yardage Pro 400 Laser Rangefinder. With these data we calculated the average length of an interval by dividing the transect length by the number of intervals (= seconds) in the transect. We also evaluated the effect of mean interval length on the mean number of plant species recorded in each transect using linear regression to determine if variation in boat speed influenced the number of species that were recorded for each transect.

To further evaluate the reliability of this new procedure, we recorded one transect 10 separate times. The transect was located just downstream from Fletcher's Bridge. We calculated the frequency of each species and for all vegetation for each transect. We then combined the data from all the transects and estimated frequency again. We calculated the coefficient of variation (CV) for frequencies from the 10 repeated transects. Calculations and statistical procedures are described below, except that we used X^2 (Chi-square) from a 2 x 10 contingency table to test the null hypothesis that there is no difference among the frequencies (i.e., proportions) in the 10 transects.

Upon returning to the laboratory at Davis, the videotapes were played back with a video recorder (Sony, Stereo Cassette Recorder Model EV-C100) capable of pausing at each second within a transect. The picture on the screen at each second was treated as a transect interval and the presence/absence of a particular species was recorded. We only considered plants in the center of the picture, since changes in water depth influence the width of the field of view. The frequency of each species was calculated according to equation 1:

Eq. 1 $f_j = j_i/k$

where f_i is the frequency of species i; j_i is the number of intervals containing species i; k is the total number of intervals within each transect or river section. Although we recorded the presence / absence of species for each interval, only values from every other interval were used to calculate species frequencies, the proportion of samples in which a species (or all species) occurs.

We used 2 x 2 contingency tables to test the hypothesis that observed and expected frequencies are similar. We compared the frequency of aquatic plants in four river sections for two years using 2 x 2 contingency tables (PROC Frequency, SAS Institute 1990). We used Fishers' Exact Test because it is recommended for 2 x 2 contingency tables. We used procedures appropriate for proportions to calculate 95 % confidence limits for aquatic plant frequency.

Results: Figure 1A shows the frequency of submersed plants that were present in the transects, from each of four river sections, sampled in late July during 1996 and again in 1997. As a group, submersed plants were more abundant in sections A and D. The middle sections of the river (B and C) were characterized

by somewhat lower plant abundance. Based on the data from the transects, the frequency of all submersed plants was 0.51 in 1996 and 0.44 in 1997. Examination of individual species frequencies provides a more detailed view of year-to-year dynamics of species abundance (Figure 1 B and C). *Zannichellia palustris* was most abundant in river section D. In 1996, it was less abundant in other river sections. This species was absent from section B, during 1996. In 1997, *Zannichellia palustris* was still most abundant below Spring Creek Bridge, but its abundance had decreased slightly. *Zannichellia palustris* abundance was not significantly different for the two years in section A, it increased in the two middle sections and declined in section D. *Elodea canadensis* was found throughout the river in 1996 and 1997. However, there were significant declines in all river sections in 1997. Large beds of *Elodea canadensis* were present in section C, near the site known as Fish Camp and at a second location just upstream from the influx of Spring Creek. In 1997, the abundance of *Elodea canadensis* was reduced at these sites and it was also apparent that sandy sediments had accumulated in these areas.

Contingency table analysis indicated no statistically significant differences among the 10 repeated recordings of the same transect. Coefficients of variation (CV) for the repeated transects were from 3.2% to 19.5% with the largest values associated with the least abundant species.

We found that the average boat speed for the 53 transects collected in 1997 was 0.6 m / s with a CV of 20%. This variation in boat speed directly influenced the length of each interval within a transect, since each second on the videotape was an interval. However, there was no evidence that this slight variation affected the average number of species per interval. The regression of number of species per interval versus interval length was not significant (P = 0.11) and interval length explained little of the variation in the number of species per interval (R-square = 0.048). It appears that slight variations in boat speed did not result in a significant bias in the frequency data gathered by these techniques.

Using the techniques described above we were able to record data from > than 50 transects in less than two days in both 1996 and 1997, with only about eight hours actually spent on the river. The most labor-intensive aspect of this procedure was viewing the videotapes which required about 50 hours of viewing time. Advantages to using videotapes are 1) the tapes can be viewed repeatedly if there is some doubt as to the species identification or whether it should be considered as under the transect line and 2) this technique can be used when certified SCUBA divers are unavailable. We were also impressed by the sharpness of the videotaped plant images. The majority of these transects were taken in water depths between 2 and 3 m. We were able to discern short-statured *Callitriche hermaphroditica* (generally < 15 cm) as well as taller plants such as *Zannichellia palustris* (present in Fall River as plants ranging from 15 cm to > 150 cm). In one transect taken in an area with 5-m deep water, *Zannichellia*

palustris, trailing near the bottom due to the current, was visible. This technique may be particularly suited to rivers, where flow results in canopy movements, which allow understory plants to be recorded. One limitation of using this technique is that the diameter of the field of view (i.e., area) recorded on the videotape changes depending on the water depth over the bottom being recorded. This can be overcome by considering only plants directly in the center of the image as being part of the transect. One way to facilitate this is to place a piece of tape on the inside of the underwater viewer. This marks the center of the field of view and represents the physical transect line. Another potential limitation is water clarity. However, this was not a problem at the time these transects were made in Fall River, which is very clear throughout summer and autumn (mean light transmission through 1 m of the water column was 46%, N=127, mean turbidity was 0.8 NTU, N=41).

SEASONAL VARIATION OF TISSUE CARBON, NITROGEN, AND TOTAL PHENOLIC ACID CONTENT OF EURASIAN WATERMILFOIL IN NORTHERN CALIFORNIA.

Reporting Scientist:	D. F. Spencer
Associated Technician:	G. G. Ksander

Objective: Eurasian watermilfoil *(Myriophyllum spicatum* L.*)* is an important introduced aquatic weed in North America, where it is found from Florida to Quebec in the east, and California to British Columbia in the west. Its strong competitive abilities allow it to displace native species, and its abundant growth impedes water use for agricultural, environmental, and recreational purposes. Current management techniques include mechanical and chemical methods. Recently, a native weevil *Euhrychiopsis lecontei* (Dietz), found in the northern and eastern states in the U. S., has been suggested as a possible biological control for this weed. Eurasian watermilfoil occurs in California, but *E. lecontei* does not. So, the nature of the interaction between *E. lecontei* and Eurasian watermilfoil under California conditions is unknown. Additionally, most studies on the ecology of Eurasian water milfoil have been conducted in the eastern U. S. (Smith and Barko 1990) and there is little published information on environmental conditions or plant characteristics from western U. S. habitats that support growth of Eurasian watermilfoil. Thus, as a first step toward evaluating *E. lecontei*'s potential as a biological control agent, we measured environmental and plant characteristics for Eurasian watermilfoil growing at two sites in northern California. We report here the results of those efforts.

Materials and Methods: The two sites were a shallow (1.5 m deep) pond located at the USDA-ARS Aquatic Weed Control Research Laboratory in Davis, California and a portion of the Truckee River between Tahoe City and Alpine Meadows in the Sierra Nevada Mountain Range (California). Voucher specimens of plants from these sites were identified by experts (S. Aiken, B. Hellequist, and J. Madsen) and are deposited at the UC Davis Herbarium. At approximately two-week intervals, between May, 1994 and June, 1996 we collected plant samples from the Davis pond. The top 30-cm portions of 10 shoots were freeze-dried and the carbon (C) and nitrogen (N) content determined using a Perkin-Elmer model 2400 CHN analyzer with acetanilide used as the standard. Because plant secondary compounds may play an antiherbivore role, portions of shoot tissue were also analyzed for total phenolic acids using KTioxalate as descried by Hendry and Grime (1993). Water temperature was measured at 0.5 h intervals with a datasonde (Hydrolab Corporation, Austin, TX) installed in the center of the pond. Beginning in May, 1996, watermilfoil samples were collected at three sites in the Truckee River between Tahoe City and Alpine Meadows at approximately monthly intervals, except during periods when the area was inaccessible, due to snow accumulation. Plant samples were analyzed as described above. Water temperature data for the period 1990 to 1998 were obtained from the USGS

monitoring site at Tahoe City, California. This data set consist of monthly measurements made between 8 to 12 PM.

Results: Mean milfoil tissue C over all samples was higher for plants from the Davis pond than for plants from the Truckee River (Table 1). In the Davis pond tissue C was greatest during January through March and declined gradually after that. Although early season data are lacking, tissue C for Truckee River plants appeared to display a similar pattern. Overall mean tissue N was 12% lower for plants growing in the Truckee River than for those growing in the Davis pond (Table 1). Seventy-five percent of all N measurements were less than 2.43% for the pond population and less than 2.11% for the Truckee River plants. Patterns of seasonal fluctuation in tissue N were also different for plants from the two sites. For Davis plants, tissue N was highest between January through March, declined to its lowest point in April through June and increased slightly during summer, Tissue N for Truckee River plants was greatest in April through June and declined sharply after that. Compared to Truckee River plants, tissue N was generally greater during summer for watermilfoil growing in the Davis pond.

Phenolic acid content of Truckee River plants was similar to that of plants from the Davis pond (Table 1). Phenolic acid content of Davis plants was positively, but weakly, correlated with tissue C. For Davis plants, phenolic acid content was greatest during the winter (November through March) and was considerably less during the rest of the year. Seasonal changes in phenolic acid levels for Truckee River plants were different. Limited data from these plants indicated that phenolic acid content was lower in December, than at other times during the year.

Mean monthly water temperature for the Truckee River site was less than 20 °C for the eight-year data set. Warmest water temperatures occurred in August and September at this site. Using this data set we, estimated accumulated degree- days for the Truckee River. Given the 309 degree-day requirement for development from egg to adult for the watermilfoil weevil, data indicate that just over two generations of weevils would develop in the Truckee River. During a 29-month period beginning January, 1994, mean monthly water temperature for the Davis pond exceeded 20 °C , only during July and August. However, because the water in the pond is warmer earlier in the year, the accumulated degree days is considerably greater in this habitat. From three to six generations of weevils should be able to develop at this site depending on year-to-year variation in temperature. Temperature differences for the pond and river sites imply that the potential biological control agent, the watermilfoil weevil, may have different developmental rates in these habitats.

These results imply that the watermilfoil weevil, should not be expected to have the same impact on watermilfoil populations at all sites in northern California and that the variable impact may be partially attributable to differences in plant quality and water temperature. These results also underscore the importance of obtaining data on the relationship between food quality and development rate for the watermilfoil weevil. Information of this type can be used

to design ecologically realistic experiments for assessing the performance of watermilfoil weevils as biological control agents.

Table 1. Carbon (C, %), nitrogen (N, %) and total phenolic acid content (μM/g) for two populations of Eurasian water milfoil growing at two sites in northern California.

Parameter	C (%)		N (%)		Phenolic Acid (μM/g)	
	Truckee River	Davis Pond	Truckee River	Davis Pond	Truckee River	Davis Pond
N	65	668	65	668	65	509
Mean	33.83	37.67	1.82	2.06	165.7	167.5
Std. Deviation	4.87	3.02	0.51	0.54	78.3	113.3
Maximum	39.62	43.13	3.74	4.53	317.1	713.5
Minimum	18.45	19.54	1.03	0.7	3.6	17.56
75% Quantile	36.73	39.70	2.11	2.43	222.8	223.42
Median	35.07	38.20	1.74	2.00	166.3	138.4
25% Quantile	33.41	36.36	1.43	1.66	119.5	83.9
Mode	36.59	36.53	1.39	1.66	119.5	23.6

VERTICAL DISTRIBUTION OF BIOMASS IN EURASIAN WATERMILFOIL (*Myriophyllum spicatum*) GROWTH WITH COONTAIL (*Ceratophylum demersum*) IN FOUR DEPTHS

Reporting Scientist: Anderson, Lars

Associated Technician: Pirosko, Chris

Objective: *Ceratophyllum demersum* is a native submersed aquatic plant found quite commonly throughout the western states. However, it can produce problematic populations with high biomass that interferes with water storage, recreational uses and conveyance of water. *M. spicatum* is an exotic species that is often found *with C. demersum* and other native plants, and often out competes them. *C. demersum* has no roots, whereas *M. spictum* has an efficient root systems that allow it to anchor in flowing water and extract nutrients from sediments. This study was conducted to compare the growth responses of the two species to different water depths, and thus different light fields when all other conditions are not varied (e.g. temperature, water quality, water-borne nutrients).

Methods and Materials: Both species were obtained from the shoreline at the Tahoe Keys Marina at Lake Tahoe on 8/20/97 and brought to the Aquatic Weed Facility at UC Davis. Sediment from the collection areas were also obtained. 15 cm shoot tips and root crowns of *M. spicatum* were planted in ca. 2 l plastic containers with sediment. Likewise, 15 to 2 cm shoots of *C. demersum* were inserted in sediments in separate containers. Planted containers were then arrayed randomly in circular manner in 22" dia. translucent cylinders maintained at 0.4, 0.8, 1.2, and 1.6 m water depth. Each cylinder contained three containers of each species. All cylinders were supplied with a common source of re-circulated, filtered well water that passed through a sump for temperature control. Lighting was provided by metal halide lamps adjusted to give equal irradiance at the surface of each cylinder. In this way, natural attenuation of light and associated changes in light quality were established depending the depth of water. The PAR was measured with a LiCor SPQA 2081 meter at 20 intervals before planting and 14, 28, 42, 56 and 84 days after planting (DAP). Shoot height was measured periodically and a final harvest was conducted by separating biomass at 20 cm intervals from the top of the cylinders to the bottom.

Results: Light measurements prior to plants showed typical attenuation that resulted in ca. 800 μmol m^{-2} sec^{-1} the surface to < 200 at the bottom of the 1.6 m cylinder (Figure 1). At 84 days after planting (DAP), reduction in irradiance was pronounced due to shading from the biomass produced in all cylinders. However, the greatest reductions occurred between ca. 40 cm depths to the bottoms of the 1.2 and 1.6 m cylinders, where the light field was less than 25% that at the start of the experiment. Shoot lengths were similar for both species except in the 0.8 m and 1.6 m depths where *M. spicatum* produced long shoots by 56 DAP (Fig. 2). The most striking differences are the dominance of *M.*

spicatum biomass at most vertical positions (Fig. 1) except the deepest zones (eg. 140 to 160 cm). Even at the deep area, neither plant seems to dominate as much as *M. spicatum* does at the mid-level zones. It is also clear that root production is reduced in the deeper cylinders, a pattern similar to overall biomass in this plant relative to water depth. In the shallowest conditions (0.4 m) root biomass was about equal to shoot biomass. The ability of *M. spicatum* to easily dominate mid-depth zones, coupled with it access to sediment-borne nutrients may explain its predominance in the Tahoe Keys, even though there is a mixed assemblage of both species present. This may be due to larger leaf area (overall greater surface area) or other factors. However, it seems that both species can easily occupy zones of low light.

Figure 1. Irradiance reduction and vertical distribution per plant of *M. spicatum* an

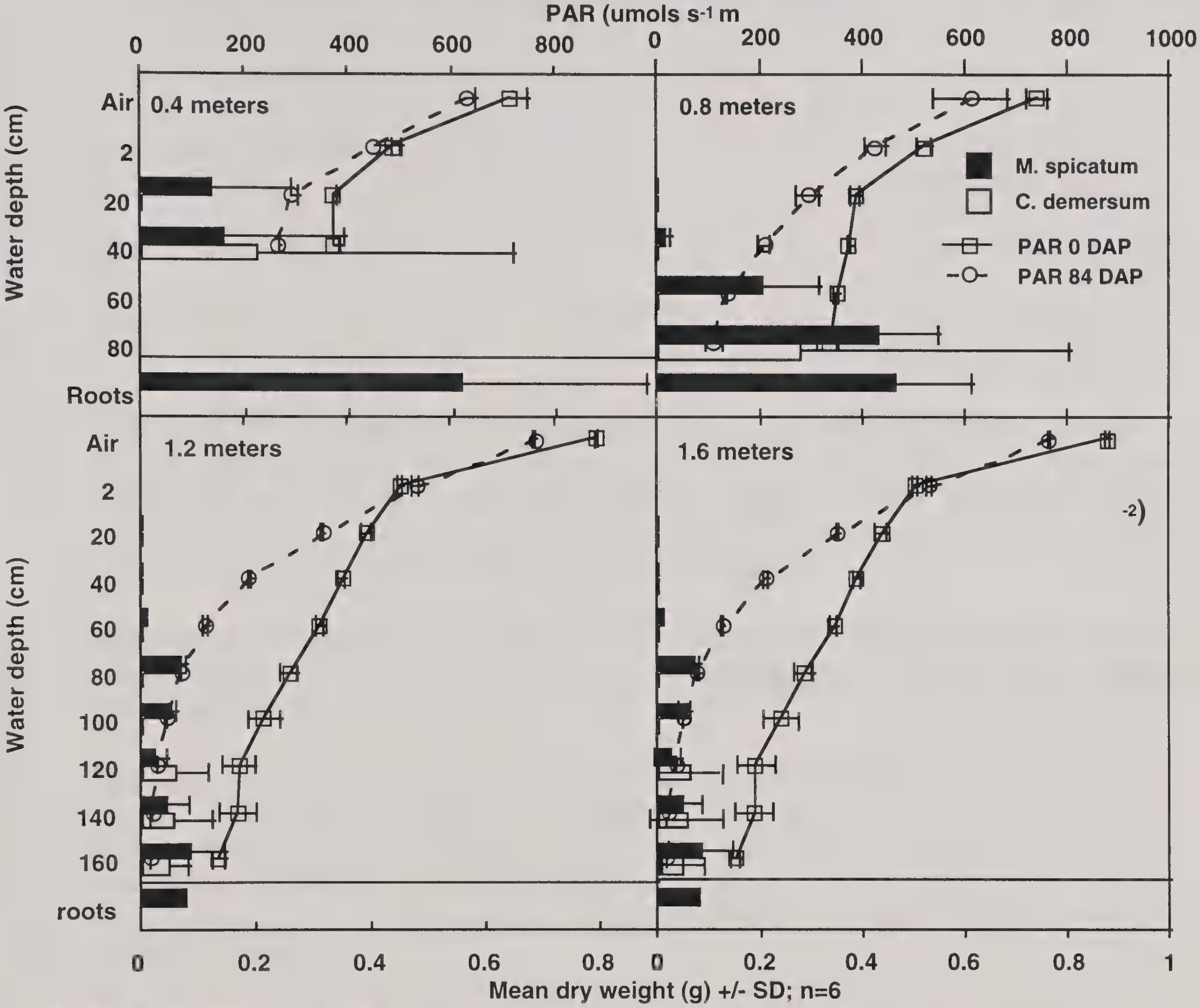
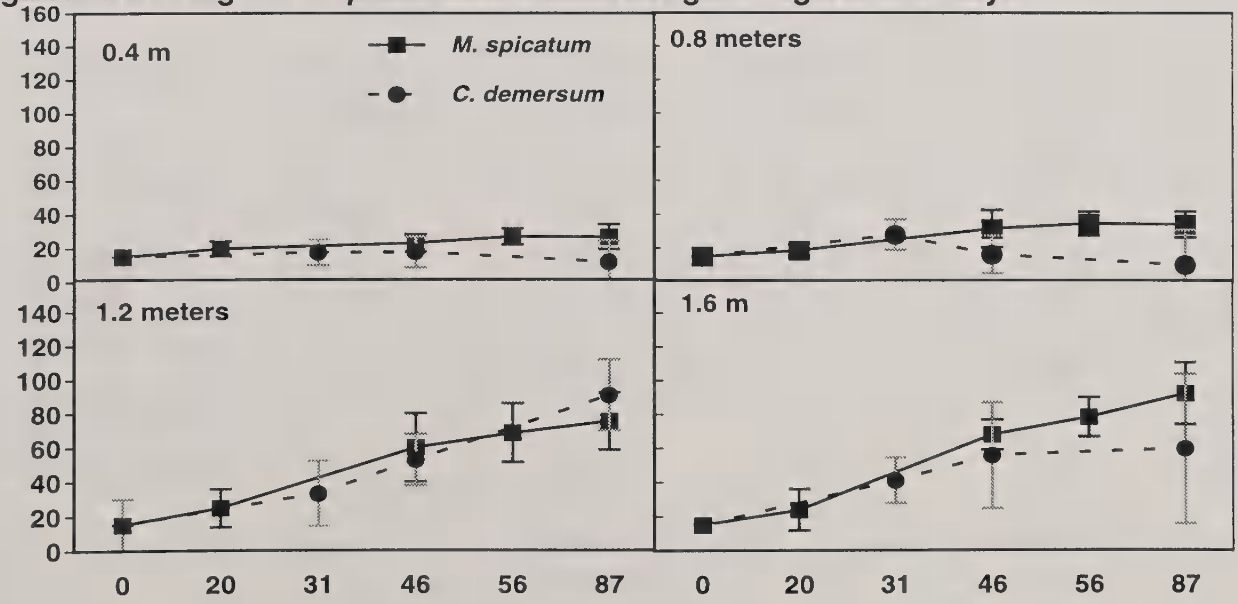


Figure 2. Shoot height of *M. spicatum* and *C. demersum* grown together for 84 days



Myriophyllum spicatum AT LAKE TAHOE: SPRING AND LATE SUMMER POPULATIONS OUTSIDE THE TAHOE KEYS MARINA

Reporting Scientist:	Anderson, Lars
Associated Technicians:	Pirosko, Chris Holmberg, Debe
Postdoctoral Researcher:	Gee, Doreen
Cooperators:	Pearce, Suzanne (TRCD)

Objective: For the past four years, this laboratory has reported on the spread of *M. spicatum* in various small marinas at Lake Tahoe. An additional survey was conducted in late June and late August last year (1997) at several sites previously know to have populations of this exotic noxious weed to determine if there had been increases in the size of the infestations.

Methods and Materials: Visual estimates of areal coverage were made through access by land to the sites listed below. Measuring tapes were used to obtain dimensions of the populations.

Results: Table 1 shows that of the six sites assessed, Meeks Bay and Crystal Shores East had increased infestations. Villa Far East appeared to have smaller infestations as did Homewood. Sites not observed, but having large populations over the past four years include: Elk Point Marina and Logan Shoals marina. If sufficient resources are available, an early fall survey will be made in 1998.

Table 1. Areal coverage of *M. spicatum* in selected Lake Tahoe Marinas

DATE:	6/27/97	8/27/97`	6/27/97	8/27/97
	Sq. Feet	Sq. Feet	Acres	Acres
Location:				
Meeks Bay	450	13,600	0.01	0.31
Obexers	21,950	22,550	0.5	0.51
Homewood	6,240	2,250	0.14	0.05
Crystal Shores West	14,076	13,140	0.32	0.30
Crystal Shores East	11,520	38,010	0.26	0.87
Villa Far East	27,160	10,065	0.62	0.23
		Total Acreage:	1.85	2.27

HYDRILLA BIOMASS AND TUBER ABUNDANCE IN THE OREGON HOUSE CANAL, YUBA COUNTY, CALIFORNIA

Reporting Scientist D. F. Spencer
Associated Technician G. G. Ksander

Cooperator N. Dechoretz

Objective: Monoecious plants of *Hydrilla verticillata* occur in the Oregon House Canal. The purpose of this study was to determine the abundance of *Hydrilla* biomass and tubers in canal sediments to serve as a baseline for assessing the impacts of management techniques.

Materials and Methods: The Oregon House Canal is located in Yuba County, California about 32 km (20 miles) north of Marysville. On October 8, 1997 (5 sites, 5 cores at each site) and August 4, 1998 (8 sites, 3 cores at each site), we collected 15-cm diameter core samples from the canal. The approximate locations of the cores are shown on Figure 1. Cores were returned to Davis, washed over 2-mm mesh metal screens to separate plant material from the sediment. Plant material was sorted by species and into tuber and non-tuber portions. The number of tubers in each sample was determined, and all plant material was dried at 80 C for 48 h. Values per square meter were determined by multiplying measured values by 58.

Results: Mean *Hydrilla* biomass and tuber density for 1997 and 1998 are given in Table 1 and Figure 2. These values may be compared to those reported by Anderson and Dechoretz (1982, Proceedings EWRS 6th Symposium on Aquatic Weeds, pp. 54 – 61) for *Hydrilla* growing in canals of the Imperial Irrigation District in southern California. They reported that standing crop was 400 to 1000 g m^{-2} which is considerably more than sampled in the Oregon House Canal. Conversely, Oregon House tuber densities are not greatly different from those observed in the Imperial Irrigation District, where tuber densities ranged from 0 to 400 m^{-2}. For the Oregon House Canal, there were significant differences between years for biomass and tuber number, however this is to be expected (especially for tuber number) since the samples were collected during different months. Previous work with monoecious *Hydrilla* indicated that tubers formed in response to decreasing photoperiods (Spencer and Anderson 1986, Weed Science 34: 551-557). Thus, the samples collected in August 1998 represent conditions during the early phases of tuber formation and those in October, conditions near the end of the growing season. Evidence that tubers were just beginning to form in the August, 1998 samples comes from the presence of geotropic shoots (68 ± 26 m^{-2}, mean ± standard error), which are precursors of tuber formation. In addition, the ratio of old to new tubers (age based on coloration) was lower for the August sample. This also suggests that new tubers

were just beginning to form. These results indicate that management techniques designed to prevent tuber formation would need to be applied prior to August. The number of tubers per gram of *Hydrilla* biomass (excluding tuber biomass) was not significantly different between years, indicating that reduced tuber abundance in the 1998 samples may have partly been a function of reduced *Hydrilla* biomass. The canal has been treated with chelated copper products during the summer of 1998.

Table 1. Characteristics of *Hydrilla verticillata* biomass and tuber abundance in the Oregon House Canal on two sampling dates. The column labeled, Pr > T, give the probability of a greater t value when testing the null hypothesis that there were no differences between years. N/A = not measured.

	Date	N	Mean	Standard Error	Pr > T
Hydrilla (g m^{-2})	10/8/97	25	50.35	6.95	
	8/4/98	24	12.32	4.41	0.0001
Nontuber Biomass[1]	10/8/97	25	5.12	0.80	
	8/4/98	24	1.19	0.33	0.0001
Tubers m^{-2}	10/8/97	25	989	133	
	8/4/98	24	250	70	0.001
Old:New Tubers	10/8/97	25	0.15	0.04	
	8/4/98	24	0.30	0.14	0.001
Tubers / g non-tuber biomass	10/8/97	25	298	73.36	
	8/4/98	24	220	63.65	0.46
Ratio Nontuber:Total Biomass	10/8/97	25	0.15	0.04	
	8/4/98	24	0.28	0.08	0.16
Geotropic Shoots m^{-2}	10/8/97	N/A	N/A	N/A	N/A
	8/4/98	24	68	26	

[1] Shoots, Rhizomes, Roots (g m^{-2})

Oregon House Hydrilla Project
Yuba County

Location of Samples
10/8/97 and 8/04/98

DEPTH DISTRIBUTION OF *HYDRILLA* TUBERS IN THE OREGON HOUSE CANAL, YUBA COUNTY, CALIFORNIA

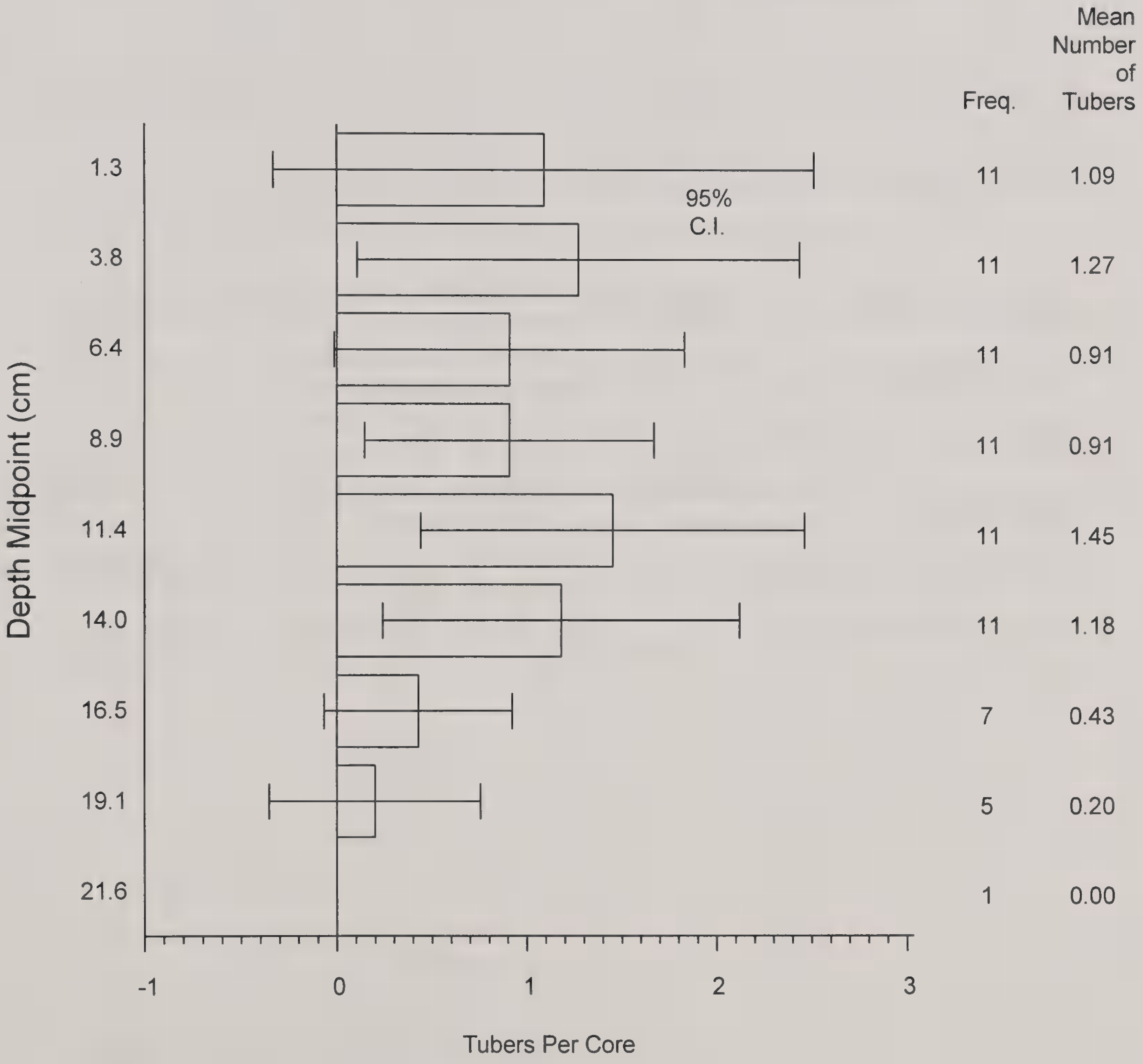
Reporting Scientist	D. F. Spencer
Associated Technician	G. G. Ksander
Cooperator	N. Dechoretz

Objective: Monoecious plants of *Hydrilla verticillata* occur in the Oregon House Canal. The purpose of this study was to determine the abundance of *Hydrilla* tubers at different depths in canal sediments. This information will serve as a baseline for designing treatment methodologies and assessing their impacts.

Materials and Methods: The Oregon House Canal is located in Yuba County, California about 32 km (20 miles) north of Marysville. On October 22, 1997, we collected 11, 10-cm diameter core samples from the canal. Cores were returned to Davis, sliced into 2.5 cm deep sections, and each section was washed over 2-mm mesh metal screens to separate plant material from the sediment. Plant material was sorted by species and into tuber and non-tuber portions. The number of tubers in each sample was determined, and all plant material was dried at 80 C for 48 h.

Results: *Hydrilla* tuber abundance at different depths in the sediment is shown in Figure 1. Eleven of eleven cores had tubers present to a depth of 14 cm. Over this depth range, tubers were more or less evenly distributed. Below 14 cm the abundance of tubers declined by half. The deepest tubers from these samples were found at 19 cm. No tubers were present in the one sample taken below 20 cm. This pattern of more or less evenly distributed tuber abundance over a range of depths is similar to that observed for sago pondweed (*Potamogeton pectinatus*) tubers growing in the Byrnes Canal (CA). It differs from the patterns of winter bud abundance of variable pondweed (*Potamogeton gramineus)* growing in the Byrnes Canal and water celery (*Vallisneria americana)* growing in the Potomac River (VA). For both of these species, winter bud abundance was highest near the surface and declined “exponentially” with depth. The depth distribution of hydrilla tubers in the Oregon House Canal indicates that management techniques designed to affect tuber survival need to be effective at depths up to 20 cm in the sediment.

Tuber Abundance vs. Depth in Oregon House Canal, October 22, 1997



PREDICTING EMERGENCE OF *HYDRILLA* FROM CLEAR LAKE SEDIMENTS

Reporting Scientist — D. F. Spencer
Associated Technician — G. G. Ksander

Cooperator — N. Dechoretz

Objective: Studies conducted outdoors at the USDA Aquatic Weed Laboratory indicate that emergence of monoecious *Hydrilla* plants from underground tubers can be predicted reasonably well from accumulated degree days. The purpose of this work was to apply the equation describing this relationship to the problem of estimating emergence of *Hydrilla* tubers from Clear Lake sediments.

Materials and Methods: We deployed 2 data loggers at 2 sites in Clear Lake. One site was near the dock of the Mosquito Control District and the second site was in Area 5. Six temperature probes were attached to each data logger. Two probes were placed on the sediment surface, two at 2.5 cm deep, and 2 at 15 cm deep in the sediment. The data loggers were programmed to record temperatures once every thirty minutes from July, 1997 to August, 1998. There were 2 sets of probes (one at each depth) at each site, and the sets were placed about 5 m apart. We calculated degree days (^{O}D) using the single triangulation method (Eqs. 1 and 2) with the lower threshold temperature being 7.8 C and the upper threshold, 27 C. The threshold values were estimated from published data on sprouting of monoecious *Hydrilla* tubers. Tuber sprouting was estimated using equation 3. This equation was derived from earlier studies.

Eq. 1 $$^{O}D = \frac{6(T_{max}-T_L)^2}{T_{max} - T_{min}} \div 12$$ (intercepted by lower threshold)

Eq. 2 $$^{O}D = \frac{6(T_{max}- T_{min} - 2T_L)}{12}$$ (entirely between both thresholds)

where T_L = Lower threshold
T_{max} = Maximum temperature
T_{min} = Minimum temperature

Eq. 3 Proportion Sprouting $= e^{mhlogt} / (1+(e^{mhlogt}))$

Where mhlogt= -6.57093+(.022*amh) and amh = accumulated degree days.

Results: During this study, some probes failed and had to be replaced. There was also a major flood in the Clear Lake watershed and both data loggers were submersed for a period, as a consequence. For these reasons, the records for each depth are not entirely complete. However, sufficient data were measured to provide good estimates of mean sediment temperature and its variation with depth. Figure 1 shows the average daily sediment temperature over the course of this study at the two sites. For the calculations we started accumulating degree days on February 11, which was the date when there was 0 degree days (i.e., temperature was lower than TL, see Figure 1) Figure 2 shows the predicted emergence of *Hydrilla* tubers based on the accumulated degree days. These results indicate that *Hydrilla* tubers should begin emerging in mid-April and continue emerging through early July. Employees of CDFA periodically sample for *Hydrilla* plants by casting a weed rake at various locations and recording the presence or absence of *Hydrilla* plants. Using this information for 1997, we calculated the proportion of samples that had *Hydrilla* plants. These data are also shown in Figure 2. There is very good agreement between the equation's predictions and the frequency of *Hydrilla* plants in the *Hydrilla* survey data. These results indicate that it would be most efficient to initiate "scouting" efforts beginning in the first or second week of April as *Hydrilla* are not likely to be found prior to that time, barring major changes in the processes that regulate sediment warming in Clear Lake.

Figure 1

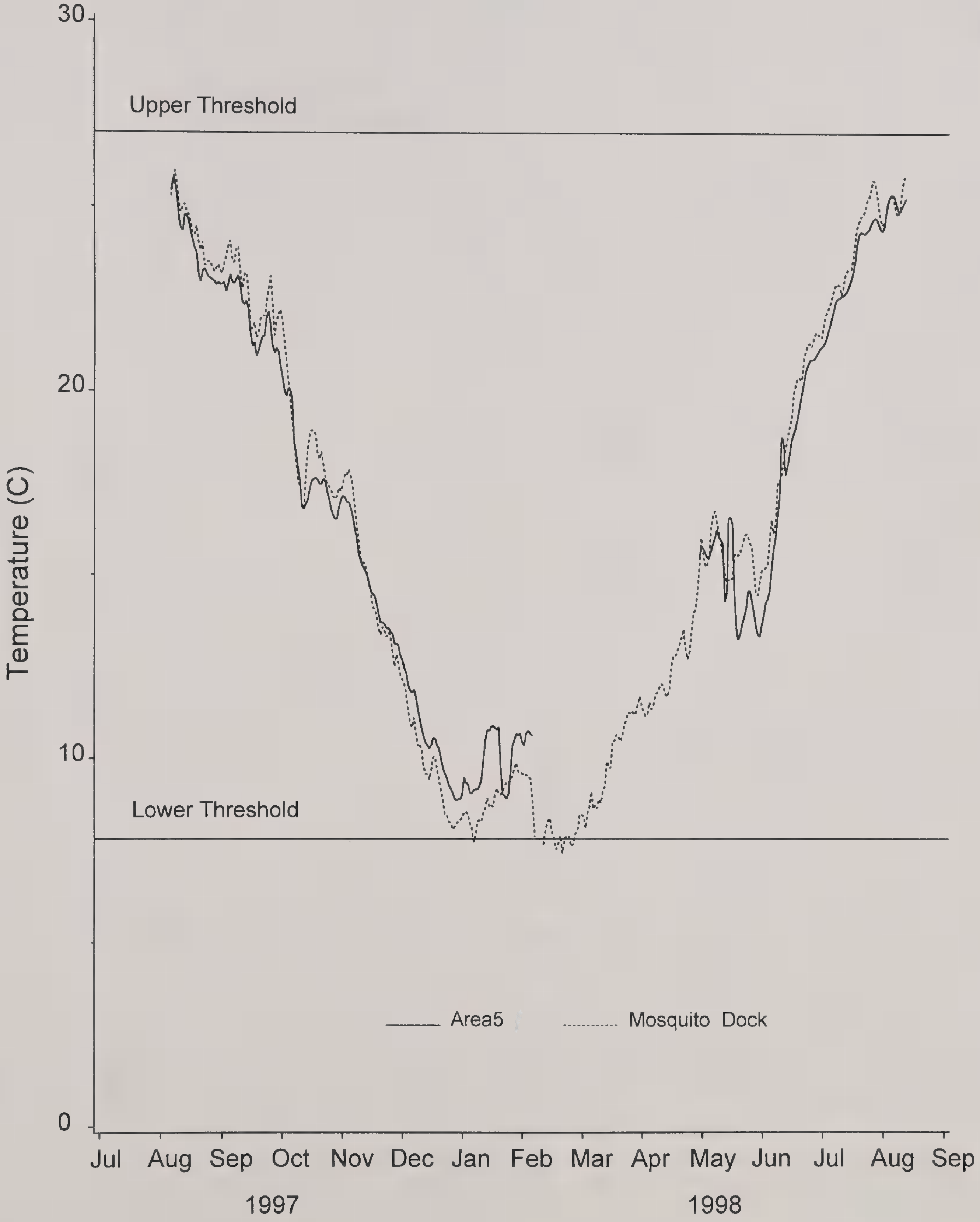
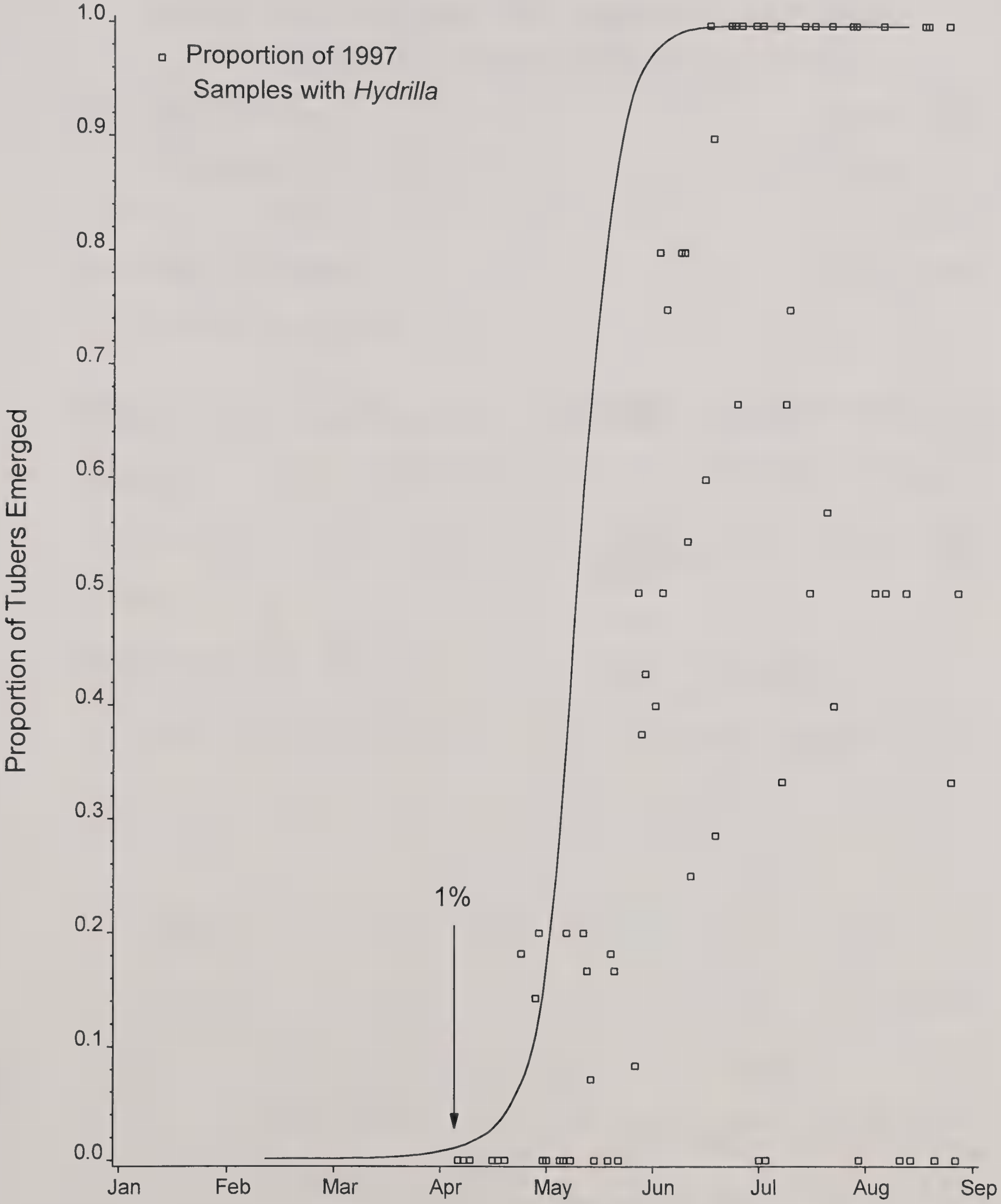

30
20
10
0
Temperature (C)
Upper Threshold
Lower Threshold
Area5
Mosquito Dock
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
1997
1998

Figure 2


□ Proportion of 1997
Samples with *Hydrilla*
1%
Proportion of Tubers Emerged
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep

SURVEY OF *Egeria densa* ACCESSIONS FOR GENETIC SIMILARITY BY RANDOM AMPLIFIED POLYMORPHIC DNA ANALYSIS (RAPDs)

NSF, Young Scholar*:	Weiss, Amy
Reporting Scientist:	Anderson, Lars
Postdoctoral Scientist:	Gee, Doreen
Associated Technicians:	Holmberg, Debe

*UC Davis Young Scholars' Program, Will C. Wood High School, Vacaville, California

Objective**:** All the *E. densa* in North America appear to be male and produce no seed because there is no sexual reproduction. *E. densa* is spread by fragmentation; almost any part of the shoot is viable and capable of yielding a new plant as long as it contains a lateral bud. Since all US *E. densa* plants are derived from clones, significant differences in genetic variation would suggest different sources for introductions. This study was done to compare genetic variation among several populations of egeria within California and other locales.

Methods and Materials: *Egeria densa* samples were collected from nine different locations around the delta, and samples were used from the UCD Botany Pond, Davis Lumber and Hardware, North Carolina, and New Zealand. Once collected, the plants were taken to the lab where the healthiest tips were removed and used for the DNA extraction. All DNA was extracted using the Qiagen DNeasy plant DNA extraction kit (Qiagen Inc.).The four primers used in this study were found effective for amplifying *E. densa* DNA in a past study comparing the effectiveness of various primers for amplification (Aung and Nijagal, 1995). A sample of *Elodea* was included in this study to verify the RAPD results; *Elodea* should produce different banding patterns because it is a different species. *Elodea* was chosen because it was found growing in the same area as one of the collected *Egeria* accessions, and because they are often mistaken to be E. *densa*.. DNA was amplified in 23 µl of master mix with 50 µl of mineral oil. The master mix contained per tube: 19.5 µl of nuclease-free water, 2.5 µl of Boehringer Mannheim PCR reaction buffer (10x) (Boehringer Mannheim Inc.), 0.5 µl dNTPs, and 1 µl of a specified Operon 10-base oligonucleotide primer (Operon Technologies, Inc.). Primers used were: OPC04, OPC19, OPR02, and OPR17. To each tube containing master mix and mineral oil, 0.76 µl of the extracted DNA was added. The DNA was quantified using the DNA DipStick Kit (Invitrogen Corp.) and found to have an average concentration of 5 ng/µl. The tubes were then placed into a Perkin Elmer DNA thermal cycler 480 (Perkin Elmer Inc.) preheated to 94° C and 0.2 µl of Boehringer Mannheim taq DNA polymerase (Boehringer Mannheim Inc.) was added to each tube. The thermal cycler was then programmed to file 7 which runs forty cycles of: 0.5 minutes at 94° C, 0.5 minutes at 37° C, 1 minute at 72° C.

After the cycles, the thermal cycler switches to file 8 which keeps the block at 10° C until the thermal cycler is turned off. Once the DNA had been amplified, the tubes were removed from the thermal cycler and 1 µl of FMC triple dye loading buffer (6x) (FMC BioProducts) was added to each tube and forced past the mineral oil. I then took 23 µl of the DNA with dye and placed it in to its designated well in the agarose gel. For the standard I used 5 µl of FMC 50-2500 bp DNA Markers (FMC BioProducts) mixed with 1 µl of the triple dye buffer, then took 5 µl of the solution and placed it into its designated well in the agarose gel. I then placed the tray into a Gel Electrophoresis Apparatus GNA-200 (Pharmacia). The apparatus was plugged into a power supply and ran on 125 volts; allowing the DNA to move down the gel with the smallest fragments running farthest and the largest staying close to the starting well position. Ethidium Bromide Tablets (Bio-Rad Laboratories), a dye that interacts with the bases of nucleic acids and fluoresces red-orange when exposed to UV light, were diluted following the directions from the company. When the DNA had run down the gel, the tray was removed and the gel was placed in 100 µl of distilled water with 50 µl of the ethidium bromide stock solution, which resulted in a concentration of 0.5 µg/ml. The gel was then soaked for fifteen minutes with agitation, after which the ethidium bromide solution was discarded and the gel was rinsed with distilled water and allowed to soak for fifteen more minutes with agitation.

The gel was analyzed on the digital imaging and analysis system (Alpha Innotech Corporation) where it was photographed and molecular weights for the bands were assigned. Photos were analyzed using a presence and absence grid to determine which accessions had the different RAPD bands, and then kept as records. Presence and absence tables were then entered as a matrix in the NT-SYS program, which calculates simple matching coefficients. Coefficients were used with UPGMA clustering to produce a dendogram showing the genetic similarity between the different *E. densa* samples.

Results: A total of 69 informative RAPD bands were scored that were produced by the four primers (Fig. 1). Comparison of the bands between accessions were recorded in presence and absence tables (Table 1). Once entered into the NT-SYS program, simple matching coefficients were created for the *E. densa* samples. UPGMA clustering produced a dendogram showing the genetic similarity between the *E. densa* accessions which is shown in figure 2.

The dendogram clearly illustrates that the *Elodea* is significantly genetically different from the *E. densa* with very few matching RAPD markers. The New Zealand accession appears to be noticeably genetically isolated compared to the *E. densa* from the delta. The *E. densa* from the delta and local aquarium stores and North Carolina all seem to be very closely related, with small variations occurring between samples from different locations.

Since the plant accessions in the Sacramento-San Joaquin delta and from various locations around North America, including the aquarium stores, have been found to be closely genetically related, and because *Egeria densa* does not sexually reproduce, it can be concluded that the *E. densa* in North America has been introduced from the same population in South America. Since the accession from New Zealand is noticeably genetically isolated, it can be concluded that the populations in New Zealand were introduced from a

different native population in South America from which the delta accessions originated.

The next step would be to compare the delta accessions to *E. densa* from various parts in South America. Which ever South American sample found to be the most closely genetically related to the *E. densa* in the delta and North America is most likely the plant population from which the North American *E. densa* originated. Researchers can then use this South American population of plants and look for a possible biocontrol agent that is effective in controlling *E. densa* . This biocontrol agent will most likely work on the accessions in the delta and North America and because the populations have been found to be genetically related.

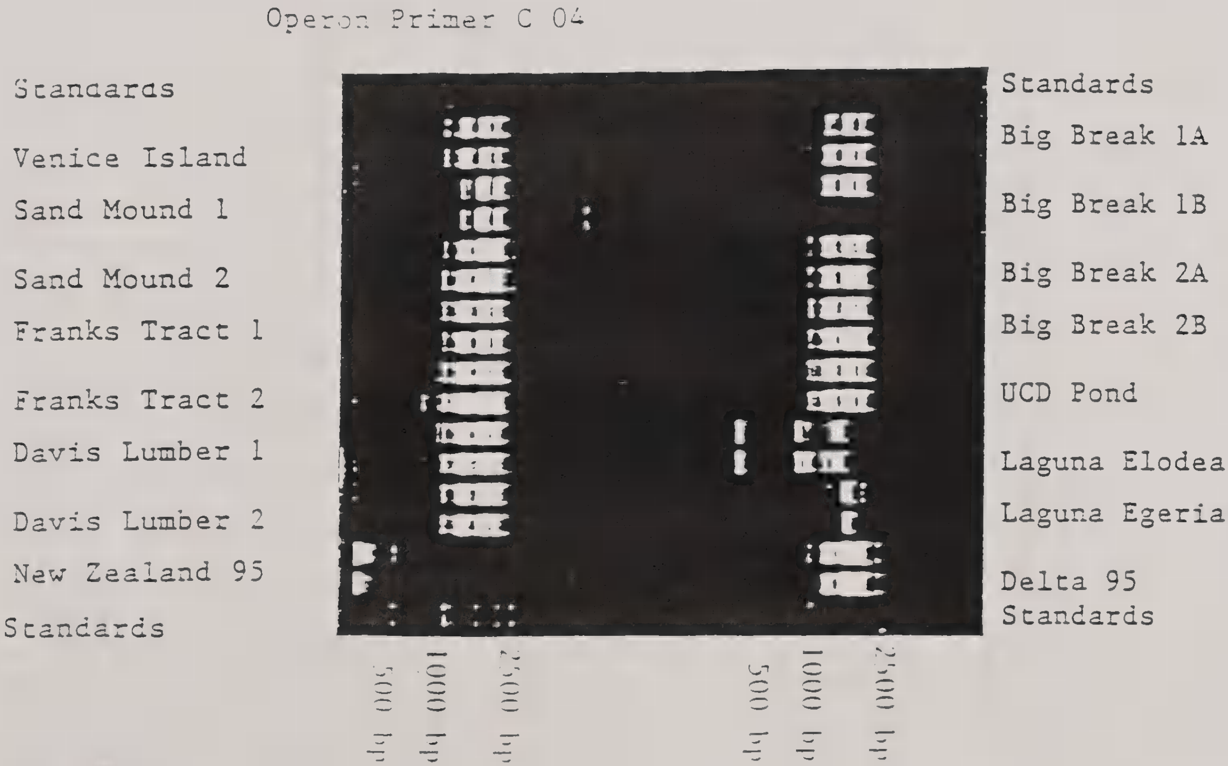


Fig. 1 A gel produced by a RAPD reaction using Operon primer C04. Collection sites and standards are as marked.

Presence/absence table for 16 *Egeria/Elodea* accessions with one RAPD primer

DNA C-19	1	2	3	4	5	6	7	8	9	10	11	12
Mw	1810	1407	1166	1072	953	884	823	617	576	466	363	281
Rf	0.39	0.45	0.51	0.53	0.56	0.59	0.61	0.69	0.71	0.76	0.82	0.87
Big Break1A	m	0	1	0	1	1	1	1	0	0	0	0
Big Break1B	m	0	1	0	1	1	1	1	0	1	0	0
Big Break2A	m	1	1	0	1	1	1	1	0	1	0	0
Big Break 2B	m	1	1	0	1	1	1	1	0	1	0	0
UCD	m	1	1	0	1	1	1	1	0	1	0	0
LW Elodea	m	0	0	1	0	0	0	0	0	0	0	0
LW Egeria	m	0	1	0	1	1	1	1	0	1	0	0
Delta ('95)	m	0	1	0	1	1	1	1	0	1	0	0
Venice Island	1	1	1	0	1	1	1	1	0	1	0	0
Sand Mound 1	1	0	1	0	1	1	1	1	0	1	0	0
Sand Mound 2	0	0	1	0	1	1	1	1	0	1	0	0
Franks Tract 1	0	0	1	0	1	1	1	1	0	1	0	0
Franks Tract 2	0	0	1	0	1	1	1	1	0	1	0	0
Davis Lumber	0	0	1	0	1	1	1	1	0	1	0	0
North Carolina	0	1	1	0	1	1	1	1	0	1	0	0
NZ ('95)	0	0	1	0	1	1	1	1	1	1	1	1
Standards	2500	2000	1500	1250	1000	700	500	400	300	200	100	
Rf	0.31	0.36	0.43	0.48	0.55	0.65	0.74	0.8	0.86	0.98	1	

Table 1. Presence/absence table for 16 *Egeria/Elodea* accessions for Operon primer C19. A total of 12 bands were scored for this primer. A 1 means the accession has the band, a 0 means the accession does not have the band, and a m means that the band is missing, but not absent from the accession.

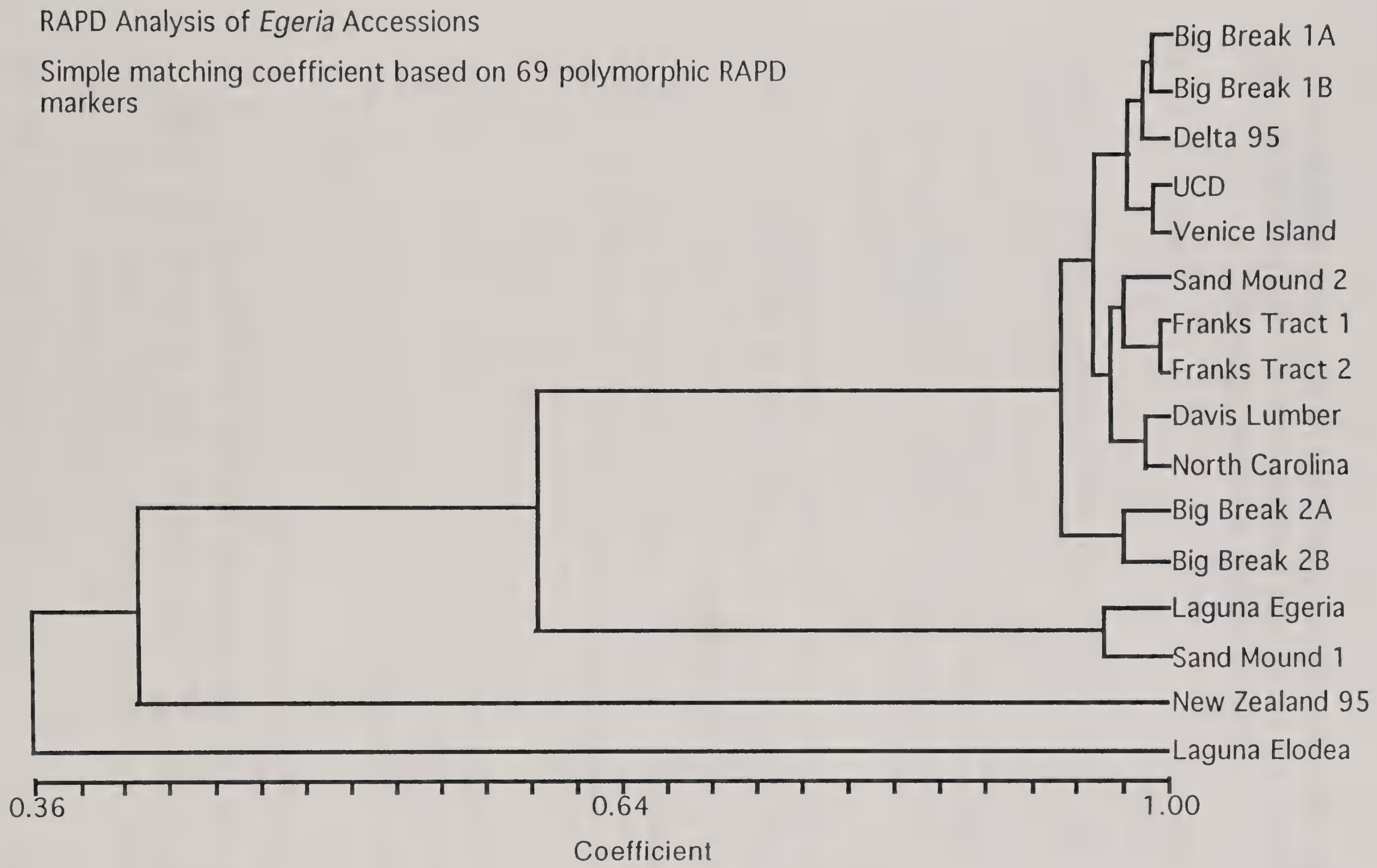


Fig. 2 A dendogram showing the genetic similarity between 16 *Egeria densa* and *Elodea* accessions analyzed through 69 RAPD markers. The coefficient shows how genetically similar the accessions are; the closer to 1, the closer the samples are genetically.

PHOTOPERIODS AND FLURIDONE AFFECT WINTER BUD DEVELOPMENT IN *POTAMOGETON NODOSUS* BY INDUCING CHANGES IN GENE EXPRESSION

Reporting scientists: Gee, Doreen
Anderson, Lars

Objective: Previous studies (Annual Report 96) showed that varying photoperiods and the addition of the aquatic herbicide, fluridone, influenced winter bud production in *Potamogeton nodosus* or american pondweed. In these studies, both long days and application of 60 ppb fluridone inhibited the induction of winter buds while short days induced the development of winter buds. In this present study, we attempted to determine whether changes occurred in gene expression in response to fluridone or to changes in photoperiod during winter bud development.

Methods and materials: Mature *P. nodosus* plants were either left untreated (controls) or treated with 60 ppb fluridone. Half the plants were placed under short days (10 h photoperiods) or long days (14 h photoperiods). Three and 6 weeks post-treatments, the plants were harvested and the rhizome tips were collected. Total RNAs were extracted from the rhizome tips using a phenol and guanidine isothiocyanate extraction method. To look for differences in gene expression between the different treatments, total RNAs from all the treatments were compared using a molecular biology method called differential display that was described briefly in the Annual Report from 1995 (p. 15). A more detailed description is outlined in Figure A.

Differential display is a powerful technique because it makes possible the study of gene expression in systems where the yield of genetic material is very sparse. In this study, the yields of RNA from the tips were only in micrograms amounts (millionth of a gram). Differential display, however, requires only 1 μg of RNA per analysis.

As shown in Figure A, once differentially display genes were discriminated with gel analysis, DNA fragments from these genes were subcloned into plasmid vectors. Once cloned into the plasmids, these DNA fragments were amplified. To identify the genes that these fragments represented, the fragments were sequenced with Sequenase Version 2.0® from USB. The nucleotide sequences were then analyzed with the Wisconsin Package GCG database to determine if these fragments represented novel or known sequences.

The final step in this method was to verify whether these genes were indeed differentially expressed. Northern analysis was performed to confirm or refute the results. With Northern analysis, different RNA populations were compared to

determine the levels of specific fragments. Under our experimental conditions, we contrasted the RNA populations of plants grown under short days ± fluridone and long days ± fluridone.

Results: Figure 1 is a representative of an autoradiograph from a differential display analysis. The arrows labeled S8, S9, S10, and S11 indicated DNA fragments that were differentially expressed between treatments. For example, S11, was expressed only in short day control after 6 weeks and not under any other conditions. S8 was expressed under long day fluridone treatment after 6 weeks and suppressed under all the other treatments.

As of this date, 13 clones have been isolated and 11 of these clones have been analyzed with Northern analysis. Of these 11, 10 were false positive. One clone S11, appeared to exhibit different levels in all the treatments as shown in Figure 2. Sequence analysis of clone S11 resolved that this clone is 237 base pairs in length. Figure 2 is the autoradiograph from the Northern analysis of S11. The two top bands represented rRNA bands. These bands were important because they indicated that the analysis was performed with equal amounts of RNA. Therefore, differences in the levels of S11 for all the treatment were not due to differences in application of the samples. Figure 2 supported the preliminary assumption that S11 was differentially expressed between the different treatments. Scrutiny of Figure 2 revealed that short day control post 3 weeks had the highest level of this fragment.

Conclusions: These results suggest that both photoperiods and fluridone treatments induced changes in gene expression during winter bud development.

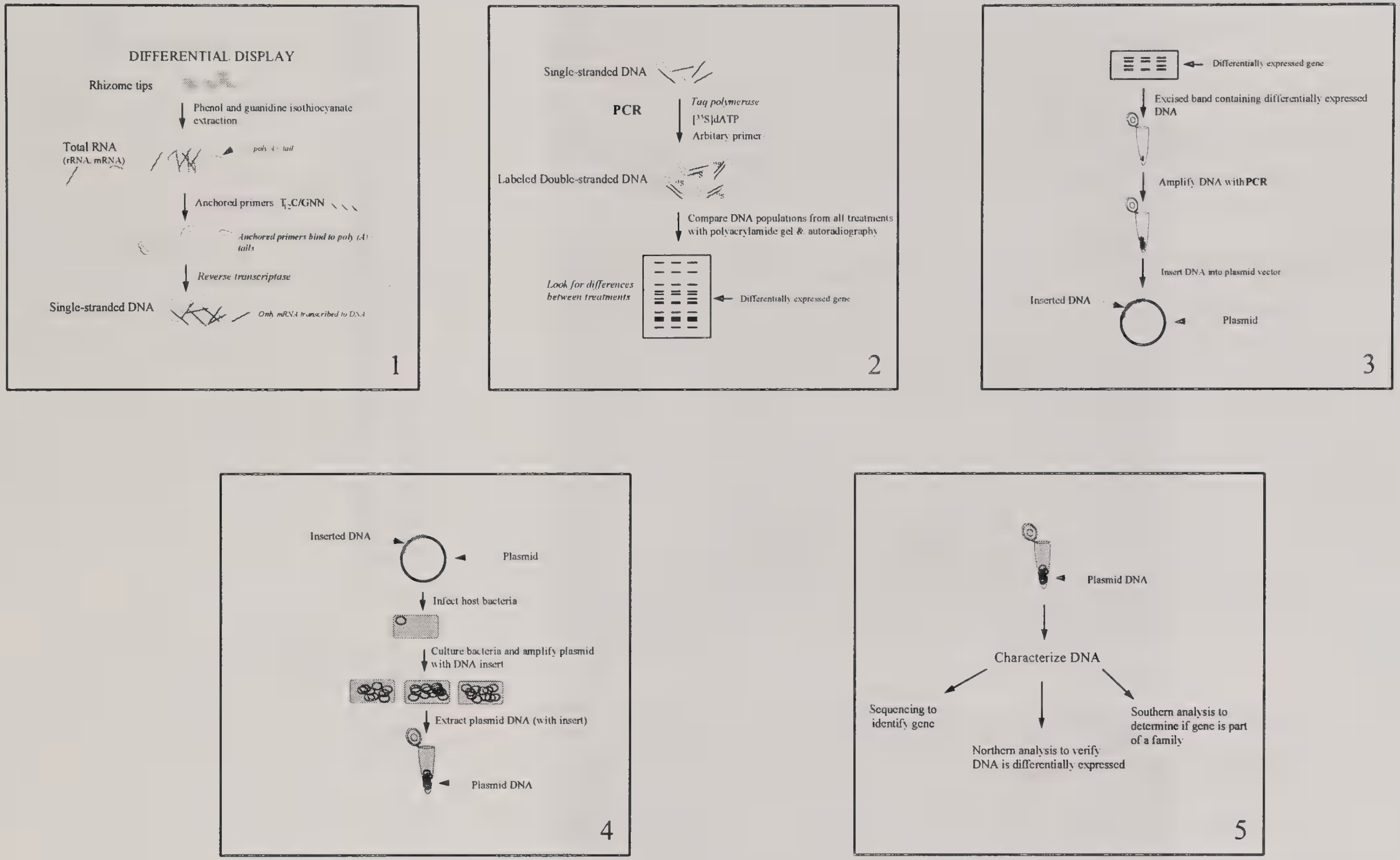


Figure A. Schematic of the differential display technique.

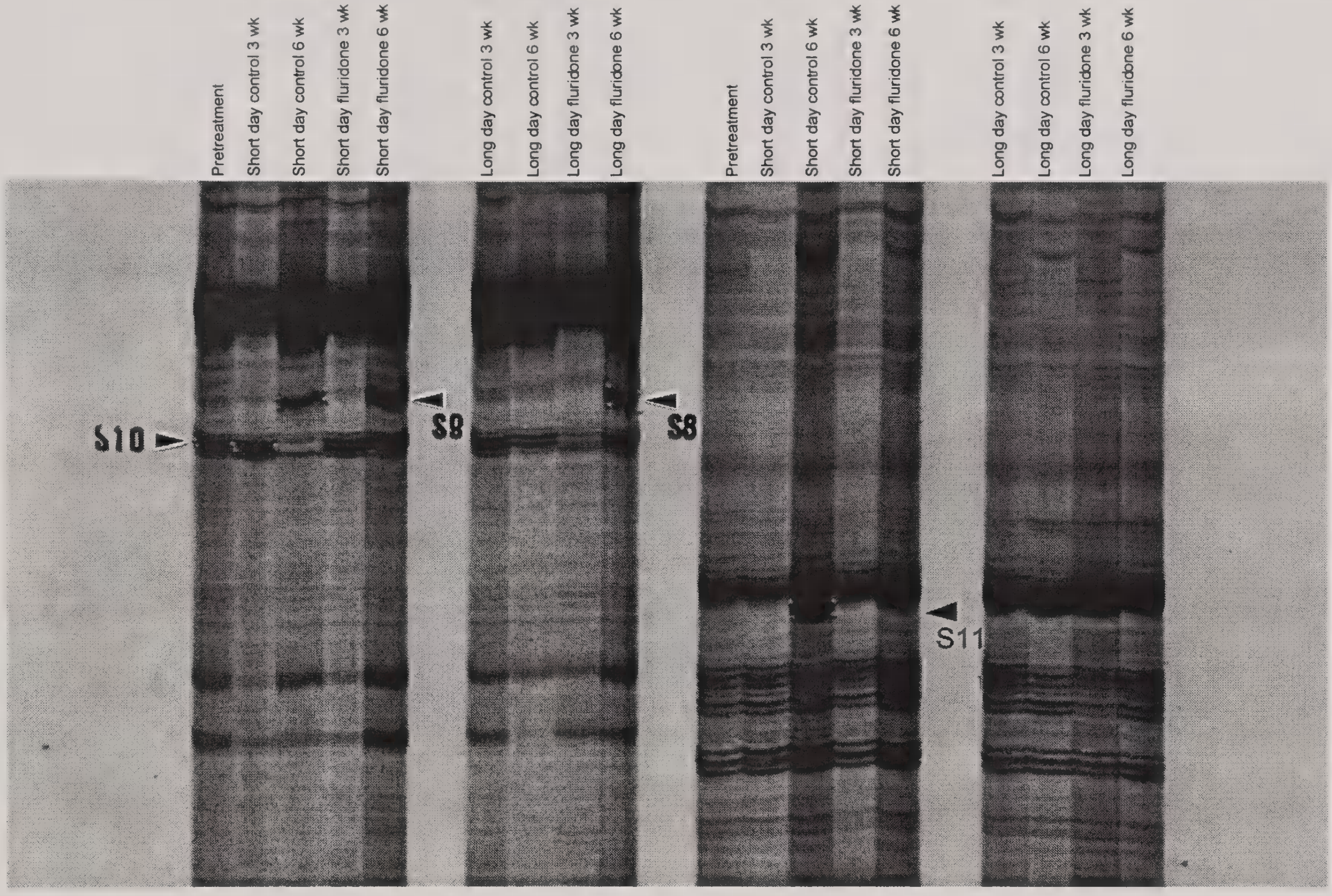


Figure 1. An autoradiograph of a differential display gel. Arrows indicate DNA fragments that were differentially expressed between the treatments.

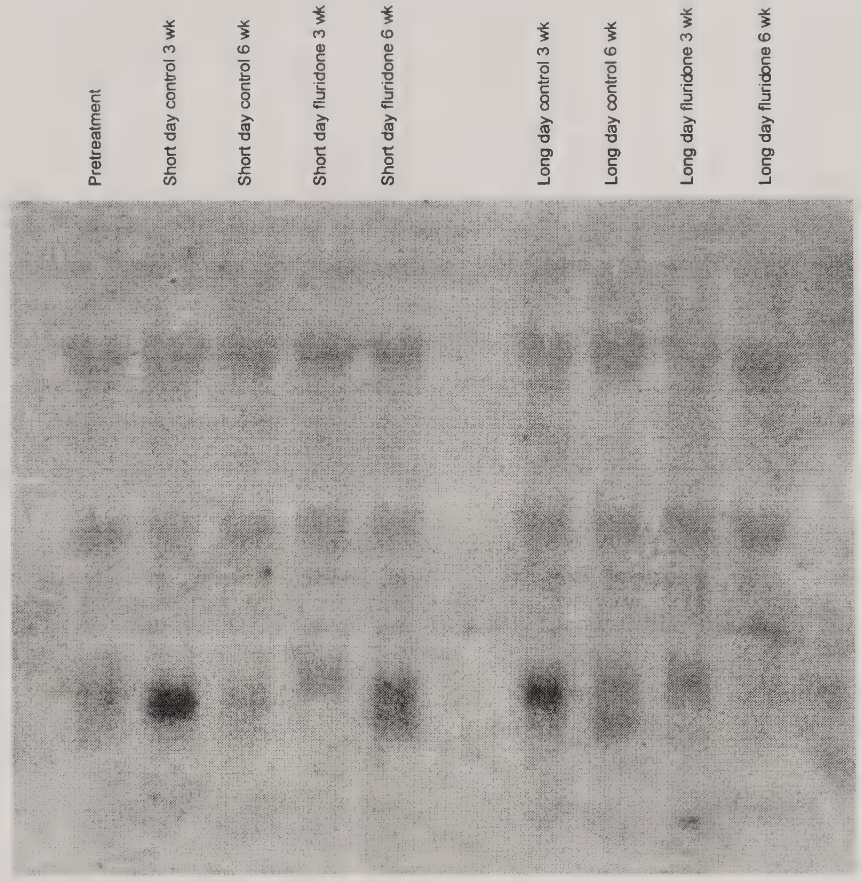


Figure 2. Northern analysis of clone S11.

ABSCISIC ACID INDUCTION OF FLOATING LEAVES IN THE AQUATIC MACROPHYTE, POTAMOGETON NODOSUS, IS ACCOMPANIED BY CHANGES IN GENE EXPRESSION

Reporting Scientists: Gee, Doreen
Anderson, Lars

Objective: The aquatic macrophyte, *P. nodosus*, impedes the flow of water in irrigation canals. A characteristic of this plant, which contributes to this ability, is the production of leaves that float on the surface of the water. These leaves differ in morphology from leaves that are formed below the surface. The induction of these floating leaves can also be stimulated by exogenous abscisic acid (ABA). For several years, we have been attempting to identify the genes that are involved with the induction of the floating leaves. The ultimate goal of this research is to institute a more efficient method of control of P. nodosus by understanding the heterophyllous changes that these plants undergo.

Methods and Materials: *P. nodosus* winter buds were sprouted for 3 days in 1% Hoagland's solution and then treated with 1 μM ABA for 12 h. Controls were incubated continuously in 1% Hoagland's solution. After treatments, the shoot tips were excised from the plants and frozen with liquid nitrogen. Total RNA was extracted from the tips using trizol® from BRL. The RNA populations from the different treatments were compared with differential display. This method is described in *Photoperiod and fluridone affect winter bud development in Potamogeton nodosus by inducing changes in gene expression* also found in this issue.

Results: Contrary to the ABA response in other experimental systems such as in embryogenesis or heat stress where the response to ABA is very conspicuous, the ABA response in leaf development is very subtle. This has been shown in this laboratory with protein analysis and with analysis of cDNA libraries constructed from mRNA from ABA-treated plants. The results from the differential display analyses have not digressed from this path. In this study, differential display has identified many transcripts that appeared to be differentially expressed between controls and ABA-treated plants. However, only quantitative differences were exhibited. Approximately 20 clones have been isolated and studied without identifying irrefutably transcripts that were induced by ABA. Research on leaf development is continuing. To introduce increased specificity to the amplifications, the lengths of the primers employed will be increased.